“There are no problems
Only solutions

John Lennon
Introduction
**Historical landmarks in oncology**

- **3,000 - 2,500 B.C.**: Hippocrates develops the theory of black bile as cause for cancer
- **460 - 370 B.C.**: Surgeon John Hunter describes how to operate on cancer
- **0 A.D.**: Emil Grubbe performs first radiation treatment
- **1871 - 1881**: Sidney Farber achieves the first remission with chemotherapy in a child with leukemia
- **1943**: First PEI treatment; official birth of the field of Interventional Oncology
- **1970**: First cryoablation treatment
- **1982**: First microwave treatment of hepatic tumor
- **1994**: Davalos publishes mathematical analysis on IRE for tissue ablation
- **2005**: 1984
- **2008**: 1988
Theodor Billroth performs first esophagectomy, laryngectomy, and gastrectomy

G.W. Fuller writes a report on the bactericidal effect of electrical pulses on water

Invention of chemotherapy after German air raid in Bari in WW II

First elective hepatic resection based on Couinaud’s segmental anatomy

First HIFU treatment

First RFA treatment of hepatic tumor

First IRE procedure performed on humans
Introduction

Cancer - A brief history

The oldest descriptions of cancer can be found in ancient manuscripts. Fossilized bone tumors and the records of Egyptian mummies provide material evidence. The oldest known account of cancer dates from approximately 3,000-2,500 B.C. It is possibly attributable to Imhotep, an Egyptian physician and architect. The papyrus describes eight cases of tumors or ulcers of the breast, which ancient physicians treated by cauterizing with a tool called the 'fire drill'. The papyrus continues the narrative by stating that “there is no effective treatment”.

Twelve centuries later, these tumors obtained their modern name – cancer. The word cancer is credited to the Greek physician Hippocrates (Kos, Greece, 460-370 B.C.). Considered the 'father of medicine', Hippocrates employed the words 'carcinos' and 'carcinoma' in his descriptions of non-ulcer forming and ulcer forming tumors. Carcinos refers to the familiar zodiac sign Cancer, the Crab. The Greeks used this term because of the tendril-like projections. Hippocrates believed that both cancer and depression developed when the four 'humors' or bodily fluids – black bile, yellow bile, phlegm and blood – fell out of balance with one another, allowing black bile to collect in excess in whichever part of the body the cancer affected. From Hippocrates onward, the humoral theory was adopted by the prominent Greek physician Claudius Galenus in the second century A.D., and by Roman and Persian physicians. This theory dominated and influenced Western medical science for the next 1300 years.

The next great wave in cancer scholarship and understanding came with the Renaissance, when scholars began to refine their understanding of the human body. Following the development of the modern scientific method in the Renaissance, scientists began to apply this to the study of disease. The Belgian physician and anatomist Andreas Vesalius (1514-1564), considered the founder of modern human anatomy, used autopsies to identify and understand anatomic structures that had previously been a mystery. No matter how hard Vesalius sought to confirm Hippocrates' theory of black bile, he failed to find this sinister porter of cancer and depression. And so, in one of the most influential books on anatomy ‘de humani corporis fabrica’ (1543), Galenus' theory of black bile as the explanation for cancer was finally dispelled.

This radical change in modern medicine was followed by Italian anatomist Giovanni Morgagni (1682-1771), who laid the foundations for scientific oncology by performing autopsies and relating the patient's illness to the pathology found after death. Scottish surgeon John Hunter (1728-1793) suggested that some cancer could be cured with surgery and described how the surgeon should decide upon which cancers to operate.

The invention of anesthesia in the nineteenth century allowed the practice of oncological surgery to flourish and physicians to develop standard surgical approaches. In 1871, the Austrian surgeon Theodor Billroth performed the first esophagectomy, followed in 1873 by the first laryngectomy and most famously, the first gastrectomy in 1881. The first pancreaticoduodenectomy was performed in 1898 by the Italian surgeon Alessandro Codivilla. American surgeon Allen Whipple refined the technique in 1935 to the procedure commonly referred to as the Whipple procedure. By the late 19th century, several surgeons also started to perform elective resections of liver tumors. However, without knowledge of the segmental anatomy of the liver, these were all based on random resections resulting in extremely high mortality rates. In 1952 Jean-Louis Lortat-Jacob performed the first elective hepatic resection that was based on the segmental anatomy described by Couinaud.
the late 1970s, the overall survival benefit of hepatic resection of colorectal liver metastases (CRLM) was established. Couinaud’s anatomic knowledge, combined with advances in anesthesia and antiseptics, resulted in an impressive reduction of complications: mortality rates dropped from around 20% during the mid-1960s to 2-3% during the early 1990s.

The twentieth century also saw the emergence of two other mainstays of cancer therapy: systemic chemotherapy and external beam radiation therapy. In 1943, a German air raid in Bari, Italy, led to the destruction of seventeen American warships. One of the ship’s secret cargo consisted of seventy-ton mustard gas bombs to be used in the battlefield. When the ship exploded, the deadly load dispersed into the air. The dissemination of the gas to the nearby harbor of Bari resulted in the death of almost a thousand people in the months following the explosion. Stewart Francis Alexander, a Lieutenant Colonel and an expert in chemical warfare, investigated the aftermath. Autopsies of the victims suggested that profound lymphoid and myeloid suppression had developed after exposure. In his report, Alexander theorized that since mustard gas all but ceased the division of certain types of somatic cells whose nature was to divide fast, it could also potentially be put to use in helping to suppress the division of certain types of cancerous cells.6 Using this information, two pharmacologists from the Yale School of medicine, Louis Goodman and Alfred Gilman, injected mustine, a related agent (the prototype nitrogen mustard anticancer chemotherapeutic) into a patient with non-Hodgkin’s lymphoma. They observed a dramatic reduction in the patient’s tumor masses.7 Although the effect lasted only a few weeks, this was the first step to the realization that cancer could be treated by pharmacological agents.8 This success was soon followed by Sidney Farber, often named the father of chemotherapy, who was the first to achieve a remission in a child with acute myeloid leukemia using the folic acid-antagonist aminopterin in 1948.8 After this discovery, an extensive search for other chemotherapeutic agents began and many different chemotherapeutics were developed. The early chemotherapy regimens were life-threatening procedures and resulted in a temporary response at best, but some of these agents are still in use. For example, fluorouracil (5-FU), still one of the mainstays of chemotherapy for colorectal liver metastases, was first described in 1957. Recently, targeted therapies such as kinase inhibitors and monoclonal antibodies have been added to the arsenal of systemic therapies.

In 1895, Wilhelm Conrad Röntgen discovered the basic properties of ionizing radiation (X-rays), and the possibility of using radiation in medicine. During early practical work and scientific investigation, experimenters noticed that prolonged exposure to X-rays created inflammation and, more rarely, tissue damage on the skin. Emil Grubbe, a medical student, hypothesized that the destruction of skin as a side effect of radiation could be used to treat tumors. On March 29th 1896, he bombarded the breast of an aged lady, Rose Lee, in which a painful recurrence after mastectomy had developed. The treatment resulted in significant tumor shrinkage. This first radiation treatment indicated the foundation of the field of radiation oncology.9 The discovery of Radium in 1898 by Marie Curie resulted in the speculation whether it could be used for therapy in the same way as X-rays. Radium was soon seen as a way to treat disorders that were not affected enough by X-ray treatment because it could be applied in a multitude of ways in which X-rays could not.10 By the 1930s, radiation oncologists were able to achieve permanent remission of several types of cancer in a significant fraction of patients. Further improvement came with the introduction of megavoltage linear accelerators in the 1950s. Nowadays, the three main divisions of radiation
therapy are external beam radiation therapy, brachytherapy and systemic radioisotope therapy. The past years, a more precise method of external beam radiation has been developed: stereotactic ablative radiotherapy (SABR). SABR refers to highly focused radiation treatment, delivering an intense dose concentrated on the tumor with sub-millimeter accuracy, while limiting the dose to the surrounding organs. SABR is increasingly used to treat lung, liver, brain and pancreatic tumors.

**Image-guided tumor ablation – A brief history**

Decades of intensive cancer research have resulted in continuously improved surgical, chemotherapeutic, and radiation treatments. This has led to a dramatic improvement in overall cancer survival over the past decades. However, despite the advances of surgical techniques, many tumors are still considered unsuitable for surgical resection, especially primary and secondary liver tumors; for example only 20-30% of the patients with CRLM are found eligible for surgery because of unfavorable tumor location, disease extent or insufficient hepatic reserve, and co-morbidity.\(^1\) The use of radiotherapy for liver tumors is traditionally limited due to the low tolerance of normal liver tissue to radiation, which results in radiation-induced liver disease in a significant proportion of patients.\(^2\) And although it greatly improves overall survival, chemotherapy always has a temporary effect and rarely leads to complete regression on its own.

**Percutaneous ethanol ablation**

In order to be able to treat some of these unresectable tumors, forward thinking surgeons, radiologists, and interventional radiologists started to consider and realize the potential to treat solid tumors using a completely new modality: ‘tumor ablation’, with the help of electrodes or probes inserted into tumors, delivering chemicals or energy in order to achieve local control. Historically, percutaneous ethanol injection (PEI) was the first percutaneous ablative therapy to be clinically applied in the early 80’s. Ethanol causes thrombosis and disruption of the endothelium of small blood vessels and induces cell death due to dehydration. The official birth of percutaneous interventional oncology was marked by the first papers on PEI of small hepatic and abdominal tumors and parathyroid hyperplasias.\(^3,4\) In two subsequent papers, Livraghi and Ebara and colleagues demonstrated PEI to be cheap, safe, and effective in the treatment of hepatocellular carcinoma (HCC).\(^5,6\) However, in the treatment of metastatic disease PEI proved less effective, since the heterogeneous and often fibrous nature of metastatic tumors restricts the diffusion of ethanol. For a similar reason, other injectable agents such as chemotherapeutic drugs and hot saline did not provide great efficacy for the treatment of metastatic liver disease. Different methods for ablation based on the deposition of physical energy therefore came into being.

**Radiofrequency ablation**

Of the different ablation techniques, radiofrequency ablation (RFA) is currently the most widely employed technique. While the clinical use of RFA is relatively new, the biological effects of radiofrequency currents were already recognized long before their therapeutic use was investigated. In 1891, D’Arsonval demonstrated that when radiofrequency waves passed through tissue, they caused an increase in tissue temperature.\(^7\) In 1910, the British urologist Edwin Beer described a new method for the treatment of bladder neoplasms using cauterization through a cystoscope,\(^8\) followed in 1911 by William Clark who described the
use of oscillatory dessication in the treatment of malignant tumors that were accessible for minor surgical procedures. However, presumably because of the lack of image-guidance it was not until 1990 that two independent investigators, McGahan and Rossi, used a modification of prior radiofrequency techniques to create coagulation via the percutaneous route using specifically designed needles. In 1993 this technique was used for the first time to ablate liver tumors in humans. RFA uses a needle applicator that emits an alternating electric current, which results in the generation of heat, and ultimately protein denaturation resulting in cell death. Over the past 10 years, manufacturers have designed more powerful generators, developed special programs for heat deposition, and achieved improved needle designs such as the deployable prongs, and the saline-cooled applicator, which caused less tissue charring, both considerably increasing coagulation volumes. Nowadays, RFA has reached a high level of reliability for the treatment of HCCs up to 5-6 cm in size, of hepatic metastases up to 3-4 cm and of some extrahepatic malignancies, such as lung, kidney and bone neoplasms.

High-intensity focused ultrasound

High-intensity focused ultrasound (HIFU) represents another thermal tumor ablation technique. The biological effects of ultrasound were known long before its use for diagnostic imaging was proposed. During the First World War, the French physicist Paul Langevin worked on a detection method for submarines. He reported that “fish placed in the beam in the neighborhood of the source operating in a small tank were killed immediately, and certain observers experienced a painful sensation on plunging the hand in this region”. In 1942, John Lynn was the first to use HIFU to create focal ablation lesions in vivo. In the late 1950s, William and Francis Fry developed a four-element HIFU transducer which was used for the first clinical HIFU treatments of Parkinsonism and hyperkinesis in 1958 by Russell Meyers. In the late 1980s, when ultrasound imaging became widely available, US-HIFU was intensely investigated for the ablation of liver tumors. In 1993, Hynynen and co-workers proposed the use of Magnetic Resonance (MR) for therapy guidance. The combination of MR guidance and HIFU ablation was coined MR-HIFU, and marked the beginning of a renewed interest in this treatment modality.

Cryoablation

Extremely cold temperatures have been used to decrease inflammation and to relieve pain since the time of the ancient Egyptians. In the 19th century an English physician, James Arnott, used a combination of ice and salt to produce tissue necrosis for tumors of the cervix and breast by topical application. Liquid air and carbon dioxide were subsequently employed as cryogens for the treatment of tumors, based on the principle used for air-conditioning and refrigeration; atmospheric gases warm when compressed and cool during expansion. Following many experimental studies using liquid nitrogen as cryogen, the first clinical experiences with the use of cryotherapy were reported by the late 1980s. The key development was the fusion of cryoablation with real-time image-guidance to verify the extent of treatment and to measure the size of the ice ball created by freezing. Interstitial hepatic cryosurgery initially started as an intraoperative procedure, mostly because of the large size of cryoprobes. Thanks to the subsequent development of argon-based cryoablation systems with much thinner cryoprobes and decreased treatment times, minimally invasive cryoablation techniques, including the percutaneous approach under cross-sectional image-guidance, have been introduced for - predominantly - kidney, lung and bone malignancies.
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Laser ablation
Laser ablation (or laser-induced interstitial thermotherapy) uses laser for thermal tumor destruction. The neodymium: yttrium-aluminum-garnet (Nd: YAG) laser system was initially used to treat head and neck tumors through precise surgical dissections rather than for tumor destruction. The first experimental application of laser hyperthermia for the treatment of liver neoplasms was reported in 1987. Recent improvements in laser-induced thermotherapy allow larger areas of coagulative necrosis than the earlier systems. However, the clinical acceptance of laser ablation has been limited, in part due to the technical complexity of the method requiring several fiber- placements compared to the other easier-to-perform thermal ablation methods.

Microwave ablation
Microwave ablation (MWA) is the most recently introduced thermal ablation technique. It uses a monopolar antenna causing water molecules in the tissue to vibrate at a higher frequency than with RFA. This generates frictional heat in the water molecules and leads to thermal coagulation of tissue. The first reports about US-guided percutaneous MWA for the treatment of unresectable HCC were published in 1994. Microwave energy has demonstrated several advantages over RFA: Microwaves readily penetrate through biologic materials, including those with low electrical conductivity, such as lung, bone, and dehydrated or charred tissue. Consequently, microwave power can produce continuous, extremely high (>150 °C) temperatures, which improves ablation efficacy by increasing thermal conduction into the surrounding tissue. Multiple antennas can be operated simultaneously. On the other hand, the distribution of microwave energy is inherently more difficult to control, which can lead to unintended injuries to other tissues.

Modern approaches take advantage of the vastly superior armamentarium of imaging strategies nowadays available. Advances in the technique combined with improved localization now make it possible to be much more aggressive and effective in attempting to achieve local control of unresectable primary or metastatic tumors. Ablative therapies have gained widespread attention and, in many cases, broad clinical acceptance as methods for treating focal malignancies in a wide range of tumor types and tissues, including primary and secondary malignancies of the liver, kidney, lung and bone. Each minimally-invasive ablation technique has their own advantages and disadvantages and particular applications. However, all the currently used effective ablative modalities are thermal techniques. Because these methods depend on thermal injury, they inadvertently carry some risk of damage to the adjacent extracellular environment like blood vessels and bile ducts, which can lead to serious complications. Other common complications of thermal ablation are perforation of adjacent bowel structures or the diaphragm. Another disadvantage of thermal ablation is that the extent of the treated area is difficult to control because blood circulation has a strong local effect on the distribution of heat. As a result, temperatures near large vessels decrease, which can lead to incomplete ablation of tumors located near these vessels. Due to this so-called ‘heat-sink effect’ the chance of complete ablation is effectively decreased to up to 50% for RFA near large vessels. In recent years, a new method of tumor ablation has emerged that addresses the limitations of thermal ablation: irreversible electroporation.
Irreversible electroporation - history

Electroporation is the phenomenon in which cell membrane permeability to ions and macromolecules is increased by exposing the cell to short high electric field pulses. This increase in permeability is, presumably, related to the formation of nano-scale defects - or pores - in the cell membrane, from which the term electro-‘poration’ stems. Under some conditions (e.g. extremely large field magnitude), membrane permeabilization is permanent and the process leads to cell lysis. It is in this sense of permanent permeabilization that most authors define irreversible electroporation (IRE). The primary use of IRE is to induce the death of undesirable cells without causing excessive heating. While electroporation has been studied and used for decades in the laboratory and food industry, it was only recently introduced to the field of interventional oncology.

The first work focusing on irreversible electroporation can be found in the 1898 study by G.W. Fuller, entitled ‘Report on the investigations into the purification of the Ohio river water at Louisville Kentucky’ (figure 1). In his report, Fuller describes an experiment in which multiple high voltage discharges have a bactericidal effect on a water sample, without significantly increasing the water’s temperature. Towards the end of the 19th century, therapeutic uses of electricity were frequently reported in the medical literature. A book by A.D. Rockwell reports experiments performed during the late 1800s in which “under the discharges of the Leyden jar the red corpuscles (of the blood, i.e. red blood cells) change their shape and lose their color”. This discovery likely refers to hemolysis induced by irreversible electroporation, although the underlying mechanisms of electroporation were not yet known.

It was not until the twentieth century that the phenomenon of electroporation was characterized as inducing increased membrane permeability, and that the thermal and the non-thermal effects of electrical energy were resolved. Some of the first observations distinguished the effects of electrical burns from non-thermal electrical injuries due to lightning, which are now thought to be caused by electroporation. Additionally, during the early 1900s, the concept of the cell membrane as a di-electric layer was advanced. During the 1950s and 1960s, the process of electroporation involving the permeabilization of cell membranes was further elucidated. Since the initial observation of the bactericidal effect of electricity on river water in 1898, research on water and food purification had continued. By the 1960s, commercial installations utilizing
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electrical pulses to provide non-thermal bacterial inactivation were available. This effort culminated in the seminal work of Sale and Hamilton, who described the optimal pulse variables used to accomplish the non-thermal bactericidal effect of electrical pulses, and demonstrated the underlying physiology of the changes in membrane conformation and permeability.

During the 1980s and 1990s, investigators discovered that electroporation induces cell fusion - 'electrofusion' and the transfer of DNA into cells - 'electrogenetransfer'. Investigators postulated the 'electroporation model', in which changes in the membrane lipids allowed transmembrane movement (figure 2). These initial discoveries inspired the later application of reversible electroporation, in which temporary cellular permeability is used to transport macromolecules into the cells, such as chemotherapeutics - 'electrochemotherapy', and to enhance transdermal drug delivery.

In the late 1990s, there were reports that cell death due to IRE came not only from necrosis but also from apoptosis, which may have been the first suggestion that electroporation could offer advantages as an ablative technique. However, it was not until 2005 that the connection between IRE and tumor ablation was made in a theoretical paper published by Davalos and Rubinsky. In this paper, a mathematical analysis was used to show that IRE is able to ablate a volume of tissue comparable to other ablation modalities, without detrimental thermal effects. Davalos' hypothesis was subsequently confirmed by Edd and colleagues, who demonstrated that areas of ablation produced by irreversible electroporation are clearly defined with cell scale resolution between ablated and non-ablated zones. Onik and colleagues complemented the previous findings with the conclusion that IRE spared certain areas when used on sensitive organs, such as the collagen network in the urethra. Furthermore, this study proposed that IRE may induce an immunological response that can aid with the clearing of dead tissues after treatment. Al-Sakere and co-workers conducted the first successful in vivo IRE procedure on mice tumors. Optimum results were achieved when a higher number of electrical pulses were delivered, specifically a tenfold increase from the 8 pulses used in most reversible electroporation settings. Their findings confirmed that IRE, negligible of any heating, was the factor responsible for complete tumor ablation. Further studies then sought to optimize the tissue ablation in vivo. The first data of IRE in human clinical trials were presented in 2011 by Thomson and colleagues who included 38 patients with advanced liver, kidney or lung tumors, with 69 total tumors treated. They used unipolar or bipolar electrodes, delivering

![Figure 2: Schematic illustration of IRE in a liver tumor. Left: liver containing an ovoid tumor with two electrodes. Middle: magnified view of the tumor showing movement of the electric current (yellow lines) across a cell membrane that leads to the breakdown of cell membrane integrity, the loss of cellular gradients, and the death of the cell. Right: ablated tumor, depicting its preserved adjacent vasculature and ducts.](image-url)
90 pulses of 70 μs high-voltage (1500–3000 V) direct current (25–45 A) per electrode pair. Complete ablation was observed in 66% of tumors. Fifteen of eighteen hepatocellular carcinomas had complete target tumor ablation, but in larger liver metastases (> 5 cm) local control was not obtained. Lung tumors showed no treatment response. The published experience rapidly grew since these initial studies, and the current literature suggests that central liver lesions and locally advanced pancreatic cancer may be the optimal target for IRE ablation.\textsuperscript{58–61} At the same time, as IRE was increasingly used in the clinical setting, treatment protocols were adjusted and more aggressive energy regimens were applied, with higher voltage and higher pulse number protocols. Although the preclinical pulse protocols were able to create cell death with negligible thermal effects, these new high-energy regimens have shown to be able to generate potentially harmful thermal effects.\textsuperscript{45,58,62–64} Given the fact that the underlying rationale for the clinical application paradigms of IRE are based in large part on the assumption of the nonthermal mechanism of cell death, characterization and quantification of the thermal effects of IRE is necessary to ensure both safe and effective ablations.

**Overall aims of this thesis**

This thesis describes the search towards better insight in the working mechanism of IRE and its efficacy for different clinical scenarios. Several preclinical and clinical studies will be presented to further elucidate the physical characteristics of the technique and the resulting clinical indications.

Chapter 1 provides an overview of the current clinical results of irreversible electroporation in terms of safety and efficacy. It also discusses in the current gaps in knowledge and clinical experience with the technique.

Chapter 2 represents the preclinical part of this thesis. It focuses on several physical aspects of the technique that are of great relevance for the clinical application of IRE. In this chapter, we aim to achieve better insight in the physical mechanisms of IRE through two preclinical laboratory studies. In chapter 2.1 we investigate the temperature development and subsequent distribution of thermal energy during IRE in a tissue phantom using different optical techniques. Chapter 2.2 focuses on the influence of a metal stent on the development of heat, and the effect of metal on the ablation zone in a tissue model as well as in porcine liver.

In chapter 3, 4, 5 and 6 the results of our clinical studies are presented. Because the delivery of high-voltage electrical pulses represents a specific challenge for the anesthesiologist, we evaluated our anesthesiologic experience of the first 28 patients who were treated with open or percutaneous IRE. These anesthetic challenges and required precautions are discussed in chapter 3.

Chapter 4 describes our clinical experience with IRE in the liver. At the basis of an effective technique to eradicate cancer cells lies its ability to achieve complete cell death of the tumor. Although preclinical animal studies have shown that IRE is capable to induce homogeneous areas of complete cell death in healthy animal tissue, the effects of IRE on in situ human cancer cells and the subsequent mechanism of cell death remain poorly understood. The results of the COLDFIRE-1 ablate-and-resect study provide better insight in the mechanism and extent of cell death when IRE of CRLM is performed in the clinical setting (Chapter
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4.1). The promising results of the COLDFIRE-1 study encouraged the development of a prospective trial to investigate the safety and efficacy of IRE for centrally located, unresectable CRLM. In chapter 4.2 the study protocol of the resultant and currently ongoing COLDFIRE-2 trial is presented. In chapter 4.3 the different technical and practical issues of IRE for CRLM are discussed, such as the indications, patient preparations, procedural steps and different 'tricks of the trade'. Chapter 4.4 is a case report; it describes an IRE procedure of a large hepatocellular adenoma that was considered unsuitable for resection or thermal ablation in a woman with a pregnancy wish.

Chapter 5 involves the pancreas. Since locally advanced pancreatic carcinoma (LAPC; AJCC stage III) typically surrounds the major vasculature and bile ducts, rendering the tumor unsuitable for resection or thermal ablation, local tumor destruction with IRE may prove efficacious. In chapter 5.1 the results of our prospective PANFIRE-study are reported; a study describing the results of 25 patients with locally advanced pancreatic cancer that were treated with percutaneous IRE. The primary aim of the PANFIRE-study is to investigate the safety of percutaneous IRE, the secondary aim represents the efficacy of IRE for this indication. In chapter 5.2, the ablation zone imaging characteristics on CT and MRI, and the tumor- and ablation zone volumes after IRE of the patients that participated in the PANFIRE-study are prospectively investigated. Early imaging characteristics that may be used to establish technical success and to predict treatment outcome, are identified. The use of IRE in LAPC results in apoptosis and a decrease in tumor mass, which may lead to reduction of tumor-associated immune suppression and the simultaneous release of immunogenic apoptotic tumor cell remnants. In chapter 5.3 we gather evidence of this suggested systemic immune stimulatory effect of the local IRE-mediated ablation of LAPC in the first 10 patients in the PANFIRE-study. Chapter 5 concludes with a case report in which pancreatic IRE was performed via the dorsal approach, due to an extensive network of collateral vessels, which impeded ventral electrode placement (Chapter 5.4).

Chapter 6 represents the use of IRE for so-called 'niche indications'. Chapter 6.1 reports the results of a case series of seven patients in which IRE was performed with palliative intent for locally recurring tumors within the lesser pelvis, that were unsuitable for surgical resection or thermal ablation due to invasion of or close vicinity to the lumbosacral, sciatic, or femoral nerves. Chapter 6.2 presents the case in which IRE was performed around a metal Wallstent in the common bile duct in a patient with hilar cholangiocarcinoma. Chapter 6.3 describes the case in which IRE was successfully performed for a local recurrence of thyroid carcinoma.

In the next sections, the results of these chapters are summarized and discussed and recommendations for future studies will be given.
References


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Introduction


There are things known and things unknown
and in between are the doors

Jim Morrison
Chapter 1

What do we know?
Irreversible electroporation: a novel technique for image-guided tumor ablation

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Abstract
Irreversible electroporation (IRE) is a novel, non-thermal, image-guided tumor ablation technique. The application of multiple high-voltage electrical pulses creates nanoscale pores in the cellular membrane. As a result, the cell loses its homeostatic properties and dies. As opposed to other local ablation techniques, the advantage of IRE is that it selectively destroys cells, whilst the extracellular matrix structures remain intact. Therefore, the anatomical framework providing strength to vulnerable structures like bile ducts, blood vessels, ureters and nerves is spared during IRE and tumors adjacent to these vulnerable structures can be safely ablated. Worldwide as well as in the Netherlands, several clinical trials are investigating the safety and efficacy of IRE for central liver tumors and pancreatic tumors that are unsuitable for the common local treatments (surgical resection, radiotherapy and thermal ablation). Although the long-term results are still unknown, the future of IRE seems promising. IRE may prove a valuable adjunct for the multidisciplinary approach of cancer.
Irreversible electroporation (IRE) is a novel image-guided tumor ablation technique that uses electrical energy to achieve cell death. The application of multiple short, high-voltage electrical pulses to tumor tissue disrupts the existing cellular membrane potential. This leads to the formation of small defects (’nano-pores’) in the cellular membrane, after which the cell loses its homeostatic capabilities, and dies.

IRE can be performed percutaneously or during laparotomy under general anesthesia. First, the exact tumor measurements are determined with either intra-operative ultrasound or CT. This determines the size and shape of the anticipated ablation zone and the number (2 to 6) and configuration of the electrodes (figure 1 and 2). The electrodes are placed using ultrasound or CT-guidance with an interelectrode distance of 1.5-2.0 cm. Next, for each electrode pair 90 pulses of 90 μsec and 1500 V/cm are delivered. The desired current should lie between 20-25 Amperes. If necessary, the electrical parameters can be adjusted. The ablation process can be monitored real-time with ultrasound; immediately a hypoechoic ablation zone appears that reliably corresponds to the final histologic ablation zone. If this zone does not fully encompass the tumor and a 5mm tumor-free margin, the electrodes are repositioned. Follow-up commonly exists of three-monthly (PET-) CT- or MRI-scan.

Why do we need a new technique?

The last two decades image-guided tumor ablation techniques have rapidly developed. Nowadays, several different ablative techniques that allow for the treatment with curative intent of unresectable tumors are accessible (table 1). The palliative setting also knows indications for this form of therapy. Especially thermal ablation techniques like radiofrequency ablation (RFA), microwave ablation (MWA), cryoablation and high-intensity focused ultrasound (HIFU) are frequently applied for the focal treatment of primary and secondary liver, lung and renal tumors.

However, for some unresectable tumors thermal ablation is also contra-indicated due to the anatomic localization of the tumor. Because the heat destructs both malignant tissue and healthy tissue surrounding the tumor, vulnerable structures like blood vessels, bile
Chapter 1.1

Ducts and nerves adjacent to the tumor can be thermally damaged, which can lead to severe complications. Also, the risk for incomplete ablation of tumors adjacent to large blood vessels is higher due to the ‘heat-sink’ effect, in which the blood flowing through the vessels cools off the tissue, resulting in heat loss during the ablation.¹

**Table 1. Overview of the current image-guided techniques for the local treatment of tumors**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Radiotherapy</td>
<td>Ionising radiation</td>
<td>Non-invasive, suitable for many tumor types</td>
<td>Radiation damage to surrounding structures; maximum dose per organ</td>
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<tr>
<td>Stereotactic ablative radiotherapy (SABR)</td>
<td>Chemical destruction</td>
<td>Cheap</td>
<td>Unpredictable distribution; less effective for tumors &gt;2cm</td>
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<td>Brachytherapy</td>
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<td>Chemical ablation</td>
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<td>Percutaneous alcohol injection (PEI)</td>
<td>Thermal coagulation using radiofrequency waves, laser waves, ultrasound or microwaves</td>
<td>Effective with liver, lung and renal tumors</td>
<td>Thermal damage to surrounding structures; higher chance for recurrence near blood vessels due to heat-sink</td>
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<tr>
<td>Percutaneous acid injection (PAI)</td>
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<td>Thermal ablation</td>
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<tr>
<td>Laserablation (SLTT)</td>
<td>Thermal coagulation</td>
<td>Effective with small tumors</td>
<td>Less effective than RFA, cryo-shock</td>
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<tr>
<td>Radiofrequency ablation (RFA)</td>
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<td>Microwave ablation (MWA)</td>
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<td>High Intensity Focused Ultrasound (HIFU)</td>
<td>Freezing tumor</td>
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<td>Cryoablation</td>
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<tr>
<td>Electrical ablation</td>
<td>Electrical cell membrane</td>
<td>Fewer damage to surrounding tissue, no heat-sink</td>
<td></td>
</tr>
<tr>
<td>Irreversible electroporation</td>
<td>destruction</td>
<td></td>
<td>Electrode placement relatively complex; efficacy not (yet) affirmed</td>
</tr>
</tbody>
</table>

**Figure 2:** Irreversible electroporation of a colorectal liver metastasis near the right portal vein and the central bile ducts. (left) PET-CT scan prior to IRE showing the central fluorodeoxyglucose (FDG-) avid lesion; (middle) CT-scan during IRE showing the electrodes placed in the periphery of the tumor; (right) PET-CT scan 6 months after IRE showing absence of FDG-activity.

**Which problem does IRE solve?**

Preclinical studies have shown that IRE effectively destroys all cells within the ablation zone, but that the extracellular matrix structures, mainly existing of collagen and elastin, remain relatively intact. Vessel walls, bile ducts and nerves that derive their strength and shape from
Irreversible electroporation

the extracellular matrix are therefore spared during IRE. As opposed to thermal ablation the preservation of the connective tissue allows for the safe ablation of tumors surrounding these vulnerable structures. In addition, since IRE is based on electrical rather than thermal destruction, there is no heat-sink effect. Tumors adjacent to large blood vessels can therefore be effectively ablated.

**What are the indications for IRE?**

IRE has received approval as an ablation technique for solid tumors. However, given the lack of clinical results for tumors outside the liver and pancreas, the technique is currently only indicated for the treatment of primary and secondary liver tumors that are unsuitable for surgical resection and thermal ablation due to their vicinity to vulnerable structures. IRE also seems promising in the palliative setting for locally advanced pancreatic carcinoma (LAPC). The indication for IRE should always be discussed in a multidisciplinary meeting.

**What do we know about efficacy?**

Several studies have investigated the efficacy of IRE for central liver tumors (table 2). Complete remission was achieved for 55-93% of the tumors after a follow-up period of 3-18 months. This percentage was higher (93-100%) for tumors smaller than 3 cm. In pancreatic cancer, improved survival was suggested after open IRE in combination with chemoradiation (n = 54) compared to chemoradiation only (n = 85) (20 versus 13 months). In addition, pain decreased significantly in the IRE group. Percutaneous IRE for LAPC also resulted in good local tumor control (n = 14). However, long-term follow-up results are still unknown.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Number of tumors</th>
<th>Size (cm, median, range)</th>
<th>Tumor type</th>
<th>Follow-up (months)</th>
<th>Complete remission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannon et al.</td>
<td>44</td>
<td>48</td>
<td>2.5 (1.1-5.0)</td>
<td>HCC (14)</td>
<td>6</td>
<td>94.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CRLM (20)</td>
<td></td>
<td>59.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheung et al.</td>
<td>11</td>
<td>16</td>
<td>1.9 (1-6.1)</td>
<td>HCC (11)</td>
<td>15</td>
<td>72.0</td>
</tr>
<tr>
<td>Kingham et al.</td>
<td>28</td>
<td>54</td>
<td>1.0 (0.5-5.0)</td>
<td>HCC (2)</td>
<td>6</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CRLM (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silk et al.</td>
<td>11</td>
<td>22</td>
<td>3.0 (1.0-4.7)</td>
<td>CRLM (9)</td>
<td>9</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomson et al.</td>
<td>1.1</td>
<td>4.0</td>
<td>2.8 (1.0-8.8)</td>
<td>CRLM (6)</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totaal</strong></td>
<td><strong>107</strong></td>
<td><strong>187</strong></td>
<td></td>
<td><strong>HCC (27)</strong></td>
<td><strong>CRLM (56)</strong></td>
<td><strong>Other (24)</strong></td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; CRLM, colorectal liver metastasis

**Is the technique difficult to learn?**

Theoretically, the ultrasound or CT-guided electrode placement is similar to thermal ablation, but because multiple electrodes must be placed precisely parallel with an interelectrode distance of 1.5-2.0 cm, this requires some practice. For an interventional
Chapter 1.1

radiologist with a broad experience in conventional tumor ablation techniques, several procedures under supervision should be sufficient to perform the technique independently.

**Future prospects**

There is a trend in medicine towards the less invasive image-guided percutaneous treatment of solid tumors. Given the promising early clinical results, the application of IRE is expected to further increase in the next years. Worldwide, multiple phase II and III studies are conducted on IRE for hepatic, pancreatic, renal and prostate tumors. These studies will determine whether IRE truly deserves a position within the multidisciplinary treatment of cancer.

**Where in the Netherlands?**

We expect that in the following years IRE will only be available in the Dutch academic hospitals with a multidisciplinary organ-specific team of specialists. The VUmc (Amsterdam) started performing IRE one year ago. The results of the COLDFIRE-I trial, an ‘ablate-and-resect’ study with ten patients with colorectal liver metastases, will be presented shortly. The only IRE-related complication was a transient and benign arrhythmia. Besides this study, 21 patients with tumors in the pancreas, liver, kidney and lesser pelvis have been treated with IRE. The preliminary results show few complications and promising short-term local tumor control. At this moment, two studies on the safety and efficacy of percutaneous IRE for central colorectal liver metastases (COLDFIRE-II trial) and LAPC (PANFIRE trial) are conducted in the VUmc. VUmc, AMC (Amsterdam) and LUMC (Leiden) intend to start a close collaboration with respect to research and treatment with IRE in the near future.
Irreversible electroporation

References

Irreversible electroporation for non-thermal tumor ablation in the clinical setting: a systematic review of safety and efficacy

Hester J Scheffer, Karin Nielsen, Marcus C de Jong, Aukje AJM van Tilborg, Jenny M Vieveen, Arthur (RA) Bouwman, Sybren Meijer, Cornelis van Kuijk, Petrousjka (MP) van den Tol, Martijn R Meijerink

Abstract

Objectives
Irreversible electroporation (IRE) is a novel, non-thermal tumor ablation technique that uses electrical pulses to induce cell death, whilst preserving structural integrity of bile ducts and vessels. This systematic review provides an overview of current clinical results.

Methods
All in-human literature on IRE reporting safety and/or efficacy was included. All adverse events were recorded. Tumor response on follow-up imaging from 3 months onwards was evaluated.

Results
In sixteen studies, 221 patients had 325 tumors treated in liver (n=129), pancreas (n=69), kidney (n=14), lung (n=6), lesser pelvis (n=1) and lymph node (n=2). No major adverse events during IRE were reported. IRE caused only minor complications in the liver, but three major complications in the pancreas (bile leak n=2, portal vein thrombosis n=1). Complete response at 3 months was 67-100% for hepatic tumors (93-100% for tumors <3cm). Pancreatic IRE combined with surgery lead to prolonged survival compared to control (20 vs 13 months) and significant pain reduction.

Conclusion
Where other techniques are unsuitable, IRE is a promising modality for the ablation of tumors near bile ducts and blood vessels. This review gives an extensive overview of the available evidence, which unfortunately is limited in terms of quality and quantity. With this in mind, IRE of central liver tumors seems relatively safe without major complications, whereas complications after pancreatic IRE appear more severe. The available limited results for tumor control are generally good. Overall, the future of IRE for difficult-to-reach tumors appears promising.
Introduction

In the past two decades, image-guided ablation for focal tumor treatment has received substantial attention when surgical options are precluded. The rapid development of ablative devices in the past years, has led to a continuous expansion of treatment options. Nowadays, tumor ablation has been accepted as a valuable adjunct to the traditional surgical, chemotherapeutic and radiation regimens. Different ablative techniques for the treatment of unresectable tumors are percutaneous ethanol injection, stereotactic ablative radiotherapy, and thermal ablation such as cryoablation, laser interstitial thermotherapy, high intensity focused ultrasound, microwave ablation and radiofrequency ablation (RFA).

Recently, a new treatment method with certain advantages over the existing ablative techniques has gained widespread attention: irreversible electroporation (IRE). With IRE, cell death is induced with electrical energy. Under image guidance, electrodes are placed around the tumor and through multiple short high-voltage electrical pulses, the existing cell membrane potential is disturbed (figure 1). As a consequence, nanoscale defects appear in the lipid bilayer of the cell membrane. Depending on the amplitude and duration of the pulses, the permeability of the cell membrane is reversible after which the cell survives, or irreversible after which the cell dies through loss of homeostasis. Although IRE is believed to effectively destroy all cells within the ablation zone, the non-thermal nature of IRE results in relative preservation of the extracellular matrix. As a result, the structural integrity of inlaying and adjacent tissue structures such as vessels and bile ducts remains intact. Moreover, treatment effect should not be impeded by heat-sink.

Numerous animal studies have investigated these hypotheses: indeed, the integrity of portal triad structures, bowel wall, pancreatic duct and urinary collecting system is guarded, due to sparing of the collagen scaffold, followed by regeneration. Following IRE around peripheral nerves, preservation of endoneural architecture and proliferation of Schwann cells may enable axonal regeneration with full function recovery. Most importantly, complete cell death has been confirmed throughout the ablation zone within hours after IRE, as well as significant tumor reduction of hepatic and pancreatic cancer xenografts in mice.

With these distinctive characteristics, IRE may be suitable for the treatment of tumors ineligible for surgical resection or thermal ablation due to unfavorable location. However, the local application of an excessive electric field is a potential hazard, since the pulses could induce cardiac arrhythmias and severe muscle contractions. The last two years, a growing experience with IRE in humans has been reported in the literature.

To investigate how the theoretical advantages of IRE are reflected in clinical practice, a systematic review was performed. Objectives were safety and efficacy in terms of complications, tumor response, survival and symptom reduction. The analyzed data should inform clinicians on the current position of IRE in interventional oncology, its indications for clinical use, and should provide researchers a compass for future clinical studies.

Method

The review was written according to the PRISMA guidelines for reporting systematic reviews. The reviewers agreed to the terminology suggested in “Image-Guided Tumor
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Figure 1: IRE-procedure of a centrally located colorectal liver metastasis (arrowhead). (A-C) F18-FDG PET-CT, ceCT and MRI-DWIb800 pre-IRE images showing a small segment IV avid lesion abutting the middle hepatic vein (MHV) and approximate to the common bile duct (CBD) and portal vein (PV). (D,E) Percutaneous CT guided IRE procedure with electrodes in situ. (F) MRI-DWIb800 24h post IRE showing a typical hypointense ablation zone surrounded by a hyperintense rim. (G-I) Imaging 3 months post IRE showing a vaguely demarcated hypodense scar lesion on ceCT, which becomes isointense on MRI-DWIb800 and non-avid on F18-FDG PET-CT.
A systematic review of IRE

Ablation: Standardization of Terminology and Reporting Criteria

Search strategy

A comprehensive systematic review of the literature published until November 2013 was performed using Embase and Medline (PubMed). Alternatively found studies were also checked for eligibility. MeSH search terms and keywords used in the search were: irreversible electroporation (IRE), electroporation, electroporation and electropermeabilization and electrocoagulation.

In- and exclusion criteria

Studies were included if they met all of the following criteria: (1) human subject(s), (2) who underwent IRE, (3) of primary or secondary tumor(s), (4) investigating safety and/or efficacy. Exclusion criteria were: (1) review or meta-analysis, (2) abstract only. Studies of all designs in the English, French and German language were included. Two reviewers (KN and HJS) independently performed literature search, article inclusion, data extraction and quality assessment. When necessary the corresponding author was contacted to prevent analysis of overlapping study results.

Quality assessment

The Quality Assessment Tool for Quantitative Studies checklist was used to assess the quality of the included studies in terms of study design, risk of bias, confounders, blinding, data collection methods and withdrawals and drop-outs (http://ephpp.ca/PDF/Quality%20Assessment%20Tool_2010_2.pdf). Although a dedicated assessment tool for case reports does not exist, this checklist includes valuable criteria that apply to case reports as well.

Furthermore, the level of evidence of each article was scored according to the system for assigning level of evidence from the Centre for Evidence-Based Medicine (CEBM) in Oxford, UK. The levels of evidence range from

**Figure 2:** Flow diagram of the literature search and article selection.

**Figure 3:** Flow diagram of literature search and article selection
A = Selection bias; B = Study design; C = Confounders; D = Blinding; E = Data collection analysis; F = Withdrawals and drop-outs.
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1 (strong evidence) to 5 (weak evidence). Discrepancies were resolved by consensus.

Data extraction
From each article, the clinical indication for IRE was noted. Other baseline characteristics were: treated organ and tumor type, previous treatments, ablation approach and additional surgical procedures.

For safety assessment, all adverse events during IRE, related to the direct application of a strong electric field, electrode placement, or any other adverse event were recorded, as well as all adverse events during follow-up. When mentioned by the original authors, complications were divided in IRE-related and not IRE-related, and graded according to the Common Terminology Criteria of Adverse Events (CTCAE) version 3.0.26 CTCAE grade ≥3 was considered a major complication. When not provided, the reviewers addressed a grade only if it could be clearly derived from the text, which meant that treatment as well as outcome after treatment were explicitly stated (e.g. if the authors stated “resolving spontaneously” or “requiring chest drainage”). If uncertainty remained, no grade was addressed.

Despite a lack of consensus on a standard follow-up interval regimen for imaging, a period of at least 3 months is commonly suggested the minimum to allow for meaningful efficacy analysis.28 Primary technique effectiveness was defined as the percentage of tumors successfully eradicated following the initial procedure based on follow-up imaging after 3 months;18 secondary technique effectiveness was defined as successful tumor eradication from 6 months onwards after the first treatment (successful repeat ablation included). Other outcomes reported were overall survival, local progression-free survival (LPFS) and distant progression-free survival (DPFS). Treatment effect of pancreatic ablation was specified as stable disease, local progression and/or distant progression, and significant symptom reduction after at least 3 months. Studies (or patients) with follow-up <3 months were excluded from efficacy analysis.

Results
The searches identified 232 hits in Pubmed and 353 in Embase. After removal of duplicates and exclusion based on title and abstract, the manuscripts of 26 remaining articles were reviewed. Sixteen full-text articles remained for analysis (figure 2). The articles were published between August 2010 and November 2013. Six articles were case reports and ten articles were case series. In figure 3 the quality assessment summary scores of the included studies are shown. All studies were classified as level of evidence 4. Martin et al. assessed safety of pancreatic IRE (n=27)27 and subsequently assessed efficacy (n=54).22 Since the patients in these articles overlapped, the first article was used for safety analysis only and the second for efficacy analysis only. Thomson et al assessed safety and early efficacy in 37 patients with hepatic, renal and lung tumors.22 Cheung et al later reported longer follow-up results for eleven patients within this patient group with HCC specifically.24 These eleven HCC patients were excluded for analysis from the article of Thomson et al. Similarly, Kingham et al treated 28 patients with 65 perivascular hepatic tumors.25 Silk et al later reported the results of eleven patients that had 22 peribiliary hepatic metastases treated,26 of which two patients with three tumors overlapped with Kingham et al. These two patients were excluded for analysis in the article from Silk et al.
Patient characteristics

In total, 221 patients with 325 lesions in different organs were treated: 227 hepatic tumors (n=129; 49 HCC, 57 CRLM, 23 other), 70 unresectable pancreatic adenocarcinoma (n=69; 41 head, 27 body/tail, 1 uncinate process), 17 renal tumors (n=14; 10 RCC, 4 other), 8 pulmonary tumors (n=6; all different origin), one presacral tumor (metastatic endometrial carcinoma) and two lymph nodes (n=2). The majority of the patients was heavily pretreated and underwent IRE due to tumor proximity to bile ducts, bronchi, renal pyelum, presacral neural plexus or large vessels, making it unsuitable for surgery or thermal ablation. In four studies, concurrent surgical procedures were performed during open IRE\textsuperscript{24,25,27,28} and in one study concurrent thermal ablations were performed during percutaneous hepatic IRE.\textsuperscript{26} Patient characteristics are shown in table 1.

Procedure characteristics

All procedures were performed under general anesthesia. Treatment approach was open in 42.5\% (94/221), laparoscopic in 1.8\% (4/221) and percutaneous in 55.7\% (123/221). Fourteen studies emphasized administration of muscle relaxants prior to ablation.\textsuperscript{21-34} Fifteen studies described the use of ECG gating.\textsuperscript{21-33} The heterogeneity of reporting details such as inter-electrode distance, applied voltage and resulting current, pulse duration, number of electrodes and repositionings did not allow for a detailed review of these parameters.

Safety

Due to overlapping patient series in the articles from Martin et al.\textsuperscript{21,22} adverse events were available for (194/221) patients. Not IRE-related complications were most commonly associated with open surgical procedures, as stated by the authors and are not displayed in this review.\textsuperscript{21,28} In total, 43 possibly or certainly IRE-related complications were noted. Complication rate per organ was 16\% (21/129) for liver, 19\% (8/42) for pancreas, 36\% (5/14) for kidney and 50\% (3/6) for lung. In 5/43 complications the treated organ was unknown. Most complications (28/43; 64\%) were CTCAE grade I/II. Grade III, IV and V complications (3/43; 7\%) were only reported after pancreatic IRE. For 12/43 complications, grade was unknown (29\%). Adverse events are displayed in table 2.

Mortality

There were no per-procedural mortalities. Three mortalities were reported within 3 months after IRE (3/194), each after pancreatic IRE. Of these, one death was likely related to IRE-treatment, resulting in a mortality rate of 2.3\% for pancreatic IRE (1/43). This patient had pre-existing portal vein thrombosis and underwent open IRE alongside a palliative bypass procedure. He presented with worsening ascites, hepatic and renal failure and died on day 70.\textsuperscript{21} Presumably, edema after ablation had contributed to progression of portal vein thrombosis. The other two mortalities were reported by the authors to be not directly attributable to IRE.\textsuperscript{24}

Complications related to electric pulses

Expected adverse events associated with the delivery of strong electric pulses are cardiac arrhythmias and severe muscle contractions. To prevent this, pulses are generally delivered in the refractory period of the heart, and with deep muscle paralysis. Eight arrhythmias were
Table 1: Patient characteristics.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Target organ</th>
<th>No. pt</th>
<th>No. lesions</th>
<th>Age (median)</th>
<th>Tumor location</th>
<th>Approach</th>
<th>Concurrent procedures</th>
<th>Pre/post-IRE treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannon35</td>
<td>Liver</td>
<td>44</td>
<td>48</td>
<td>60</td>
<td>100% adjacent to major vascular/biliary structures and/or organs</td>
<td>Open (14) Perc (28) Lap (2)</td>
<td>Pre: abdominal resection</td>
<td>Pre-IRE: 72% CT/RT/ablation/resection</td>
</tr>
<tr>
<td>Cheung35</td>
<td>Liver</td>
<td>11</td>
<td>18</td>
<td>71</td>
<td>7/18 adjacent to major vascular/biliary structures and/or organs</td>
<td>Perc</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>Kasi36</td>
<td>Liver</td>
<td>1</td>
<td>1</td>
<td>01</td>
<td>Portal vein abutment and adjacent bowel</td>
<td>Perc</td>
<td>-</td>
<td>Pre-IRE: CT, resection</td>
</tr>
<tr>
<td>Kingham37</td>
<td>Liver</td>
<td>25</td>
<td>65</td>
<td>81</td>
<td>57% ≤ 1 cm major hepatic vein, 40% ≤ 1 cm major portal pedicle</td>
<td>Open (22) Perc (6)</td>
<td>2 perioperative pump chemotherapy</td>
<td>Pre-IRE: 86% CT Post-IRE: 71% CT</td>
</tr>
<tr>
<td>Narayanan43</td>
<td>Liver</td>
<td>21</td>
<td>29</td>
<td>61</td>
<td>82% &lt; 0.5 cm gallbladder, liver capsule or dome of diaphragm</td>
<td>Perc</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>Niessen43</td>
<td>Liver</td>
<td>1</td>
<td>1</td>
<td>65</td>
<td>Close to diaphragm and heart muscle</td>
<td>Perc</td>
<td>-</td>
<td>Pre-IRE: RFA</td>
</tr>
<tr>
<td>Niessen43</td>
<td>Liver</td>
<td>1</td>
<td>1</td>
<td>65</td>
<td>Adjacent to a TIPSS stent graft</td>
<td>Perc</td>
<td>-</td>
<td>Pre-IRE: failed TACE</td>
</tr>
<tr>
<td>Säk44</td>
<td>Liver</td>
<td>9</td>
<td>19</td>
<td>60</td>
<td>14% &lt; 1 cm CBD, 68% &lt; 1 cm primary bile duct</td>
<td>Perc</td>
<td>8 additional thermal ablation/IRE/embolization</td>
<td>Pre-IRE: surgery 100%, CT 91%, RT 9%, embolization 27%</td>
</tr>
<tr>
<td>Bagli45</td>
<td>Pancreas</td>
<td>1</td>
<td>1</td>
<td>78</td>
<td>Pancreatic body</td>
<td>Perc</td>
<td>-</td>
<td>Post-IRE: CT</td>
</tr>
<tr>
<td>Martin46</td>
<td>Pancreas</td>
<td>27</td>
<td>27</td>
<td>61</td>
<td>15 head, 12 body/neck</td>
<td>Open (27)</td>
<td>8 partial Whipple, 13 bypass, 3 partial gastrectomy, 17 ns</td>
<td>Pre-IRE: 85% CT and CRT</td>
</tr>
<tr>
<td>Martin46</td>
<td>Pancreas</td>
<td>24</td>
<td>24</td>
<td>61</td>
<td>35 head, 19 body/neck</td>
<td>Open (52) Lap (2)</td>
<td>19 partial Whipple, 35 bypass, 9 celiac plexus block, 27 ns</td>
<td>Pre-IRE: 45% CT, 45% CRT Post-IRE: 69% CT, 19% CRT</td>
</tr>
<tr>
<td>Narayanan46</td>
<td>Pancreas</td>
<td>14</td>
<td>15</td>
<td>57</td>
<td>6 head, 7 body, 1 uncinate process</td>
<td>Perc</td>
<td>-</td>
<td>Pre-IRE: 100% CT, 73% RT, 1x Whipple</td>
</tr>
<tr>
<td>Pech47</td>
<td>Kidney</td>
<td>6</td>
<td>6</td>
<td>57</td>
<td>NS</td>
<td>Open</td>
<td>All nephrectomy 15 minutes after IRE</td>
<td></td>
</tr>
<tr>
<td>Usman48</td>
<td>Lung</td>
<td>2</td>
<td>2</td>
<td>33, 70</td>
<td>Close to pulm. arteries, lobar bronchi, azygos vein, trachea</td>
<td>Perc</td>
<td>-</td>
<td>Pre-IRE: surgery, cryoaablation, RT</td>
</tr>
<tr>
<td>Niessen49</td>
<td>Presacral</td>
<td>1</td>
<td>1</td>
<td>56</td>
<td>Presacral with infiltration of the sacral bone and nerve plexus</td>
<td>Perc</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thomson49</td>
<td>Liver</td>
<td>13</td>
<td>40</td>
<td>NS</td>
<td>Adjacent to vital structures in most patients</td>
<td>Perc</td>
<td>-</td>
<td>Standard therapy not possible/unsuccessful</td>
</tr>
</tbody>
</table>

Subtotal | | | | | | | | |
| Liver | 129 | 227 |  |  | | Open (94) | |
| Pancreas | 69 | 70 |  |  |  | Perc (123) Lap (4) | |
| Kidney | 14 | 14 |  |  |  |  | |
| Lung | 6 | 8 |  |  |  |  | |
| Pelvis | 1 | 1 |  |  |  |  | |
| Linn | 2 | 2 |  |  |  |  | |

Total | 221 | 325 | 221 |

Pt: patients; Perc, percutaneous; Lap, laparoscopic; CT, chemotherapy; RT, radiotherapy; CRT, chemoradiotherapy; NS, not specified; TIPSS, transjugular intrahepatic porto-systemic shunt; TACE, trans-arterial chemoembolization; Kasi, Kasivisvanathan. *Overlapping patient series: 21 reporting safety (n=27), 22 reporting efficacy (n=54). For safety analysis 21 is used, for efficacy analysis 22 is used.

42
Table 2: Adverse events of IRE.

<table>
<thead>
<tr>
<th>Target organ</th>
<th>Approach</th>
<th>No. pt</th>
<th>No. compl. (%)</th>
<th>Electric pulse compl. (grade)</th>
<th>Treatment site compl. (grade)</th>
<th>Other compl. (grade)</th>
<th>Intervention</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Perc</td>
<td>11</td>
<td>4 (36%)</td>
<td>-</td>
<td>4 Urinary retention (II)</td>
<td>Transient catheter</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>21</td>
<td>3 (14%)</td>
<td>-</td>
<td>Pneumothorax (I)</td>
<td>None</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>13</td>
<td>2 (15%)</td>
<td>AF (II)</td>
<td>Pneumothorax (II)</td>
<td>Cardioversion</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>9</td>
<td>3 (33%)</td>
<td>-</td>
<td>3 Bile duct occlusion (ns)</td>
<td>1 Bile duct stent, 2 NS</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perc</td>
<td>6</td>
<td>1 (1%)</td>
<td>PV thrombosis (I)</td>
<td>Portal vein occlusion (ns)</td>
<td>None</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open</td>
<td>22</td>
<td>3 (14%)</td>
<td>SVT (I)</td>
<td>Bile duct occlusion (ns)</td>
<td>None</td>
<td>NS</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>44</td>
<td>5 (11%)</td>
<td>-</td>
<td>Bilary stent occlusion (ns)</td>
<td>Neurogenic bladder (NS) Dehydration (NS)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| Pancreas     | Perc     | 14     | 4 (29%)        | -                            | Pancreatitis (II)           | None                | 34          |     |
|              | Perc     | 1      | -              | -                            | Hematoma (I)                | Nausea (II)         | 35          |     |
|              | Open     | 27     | 4 (15%)        | -                            | 2 Bile leak (III, IV)       | Perc drainage       | 21*         |     |
|              | Open     | 6      | 1 (17%)        | SVT (I)                      | 2 PV Thrombosis (II, V)     | None                | 27          |     |
| Kidney       | Open     | 8      | 4 (50%)        | -                            | Ureter obstruction (II)     | Ureteral stenting   | 23          |     |
|              | Perc     | 4      | 2 (50%)        | -                            | Adrenal ablation (ns)       | None                | 23          |     |
|              | Perc     | 2      | 1 (50%)        | 2 Pneumothorax (II)          | None                        | None                | 36          |     |
| Lung         | Open     | 4      | 2 (50%)        | -                            | Parenchymal                 | None                | 33          |     |
|              | Perc     | 1      | 1 (100%)       | -                            | Hemorrhage (II)             | None                | 23          |     |
| Sacral       | Perc     | 2      | -              | -                            | Mild parestis (I)           | None                | 23          |     |
| Lnn          | Perc     | 5      | 4 VT (II) #, SVT (I) | -              | None                        | None                | 23          |     |

Total per organ

| Liver        | 129     | 21 (16%)      | 8 (4%)                      | 26 (13%)                    | 8 (4%)                  | 43          |     |
| Pancreas     | 42      | 8 (19%)       | 8 (4%)                      | 26 (13%)                    | 8 (4%)                  | 43          |     |
| Kidney       | 14      | 5 (36%)       | 8 (4%)                      | 26 (13%)                    | 8 (4%)                  | 43          |     |
| Sacral, Lnn  | 6       | 3 (50%)       | 8 (4%)                      | 26 (13%)                    | 8 (4%)                  | 43          |     |
| NS           | 5       |              |                            | 26 (13%)                    | 8 (4%)                  | 43          |     |

Pt, patients; Perc, percutaneous; Lap, laparoscopic; CT, chemotherapy; RT, radiotherapy; CRT, chemoradiotherapy; NS, not specified; TIPSS, transjugular intrahepatic porto-systemic shunt; TACE, trans-arterial chemo embolization; Inn, lymph node. * Overlapping patient series: 21 reporting safety (n=27), 22 reporting efficacy (n=54). For safety analysis 21 is used, for efficacy analysis 22 is used.
Chapter 1.2

reported (CTCAE grade I-II), corresponding to a total incidence of 4% (8/194). Without synchronized pulsing, ventricular arrhythmias occurred four times (transient ventricular tachycardia), immediately resolving after pulse delivery abortion. With the use of cardiac synchronization only atrial arrhythmias occurred (n=4), resolving spontaneously or within 24 hours after therapy. With the administration of muscle relaxants, no uncontrolled muscle contractions were reported. Thomson et al were the only ones to report a transient increase in systolic blood pressure in all patients directly after IRE (20-30mmHg), which normalized spontaneously.

Site-related complications

No major complications were reported regarding hepatic IRE. Direct puncture-related complications were grade I and II pneumothorax (n=2), pleural effusion (n=1) and grade II hemothorax (n=1).31-33 Most lesions were located close to portal vessels or bile ducts. Stenosis or occlusion of these structures was reported in 8/129 treated patients (6%). Silk et al evaluated biliary complications after IRE of 19 liver metastases in 9 patients within 1 cm of the common, left or right hepatic duct.26 One patient showed subsegmental bile duct prominence without increased bilirubin. This still existed after 11 months, without progressive dilatation or segmental atrophy. Retrospective review of CT images showed that one needle was placed in direct contact with the bile duct. Two other patients showed bile duct dilatation with increased bilirubin, for which one required stent placement; both conditions appeared to be secondary to tumor progression. Kingham et al treated 28 patients with 65 tumors of which the majority was located less than 1 cm from a major hepatic vein or portal pedicle.25 Complications were grade I portal vein thrombosis, portal vein and bile duct occlusion (grades not provided), each after ablation within 0.5 cm from a major portal pedicle. Other complications were biliary stent occlusion and cholangitis.26 Ablation of an HCC near a transjugular intrahepatic porto-systemic shunt (TIPSS) did not cause occlusion or destruction of the shunt.32 Retrospective comparison of postprocedural pain after hepatic IRE and RFA showed similar moderate pain intensity with comparable amounts of self-administered pain medication.30

Pancreatic IRE had an overall complication rate of 19% (8/42) and major complication rate of 7% (3/42). Direct complications were a spontaneous pneumothorax during anesthesia requiring chest drainage and a small subcutaneous hematoma.34 On follow-up, five site-specific complications occurred. Two were portal vein thrombosis after open IRE; one required paracentesis and aldactone, one was fatal.21 Furthermore, two cases of bile leak (CTCAE grade III-IV) were reported after open IRE;21 one patient had undergone concurrent duodenal stent removal via duodenotomy, in the other patient the electrodes were placed transduodenally. Both complications required percutaneous drainage after which they resolved. Pancreatitis was reported only once in 42 procedures, resolving spontaneously (CTCAE grade II).21 Of note, Martin et al reported elevated amylase and lipase in all 27 patients, without clinical signs of pancreatitis.21 Abdominal pain grade I was reported in all patients (15/15) after percutaneous pancreatic ablation.24,35 Pain was always easily manageable with oral or intravenous analgesics and did not lead to prolonged hospitalization.

Besides one minor arrhythmia,27 renal IRE was complicated by accidental adrenal ablation leading to severe postural hypotension for 2 months.23 One ureter that was previously damaged by RFA required stenting after IRE, but no stricture was observed in the other six
patients in whom the ureter or collecting system was within the treatment zone. Central IRE caused transient hematuria in two patients.23

Although lung tumors were located close to pulmonary arteries and azygos vein, lobar bronchi and the trachea, only one unexpected minor complication occurred (grade 1 parenchymal hemorrhage).26 Furthermore, two of six lung ablations were associated with self-limiting pneumothorax, which is an expected event after lung ablation.21

Table 3: Efficacy of hepatic IRE

<table>
<thead>
<tr>
<th>Author</th>
<th>No. pt</th>
<th>No. lesions</th>
<th>Size (cm, median, range)</th>
<th>Approach</th>
<th>Tumor type</th>
<th>Primary efficacy (%)</th>
<th>Secondary efficacy (months, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannon29</td>
<td>44</td>
<td>48</td>
<td>2.5</td>
<td>Open (14) Perc (28) Lap (2)</td>
<td>HCC (14) CRLM (20) Other (10)</td>
<td>97</td>
<td>6 (95) 12 (60)</td>
</tr>
<tr>
<td>Cheung26</td>
<td>11</td>
<td>18</td>
<td>(1.1-5.0)</td>
<td>Perc</td>
<td>HCC (11)</td>
<td>67</td>
<td>18 (72)*</td>
</tr>
<tr>
<td>Kasivisvanathan28</td>
<td>1</td>
<td>1</td>
<td>1.9</td>
<td>Perc</td>
<td>CRLM</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Kinmonth25</td>
<td>28</td>
<td>54</td>
<td>(1-6.1)</td>
<td>Open (22) Perc (6)</td>
<td>HCC (2) CRLM (21) Ovary (5)</td>
<td>96</td>
<td>6 (93)</td>
</tr>
<tr>
<td>Silk30</td>
<td>9</td>
<td>17</td>
<td>2.0</td>
<td>Perc</td>
<td>CRLM (8) Ovary (1)</td>
<td>77</td>
<td>9 (55)</td>
</tr>
<tr>
<td>Thomson31</td>
<td>1.5</td>
<td>43</td>
<td>1.0</td>
<td>Perc</td>
<td>CRLM (6) Ovary (7)</td>
<td>77</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>183</td>
<td>(0.5-5.0)</td>
<td>Open (36) Perc (68) Lap (2)</td>
<td>HCC (27) CRLM (56) Ovary (23)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Pt, patients; Perc, percutaneous; HCC, hepatocellular carcinoma; CRLM, colorectal liver metastasis; † Follow-up results reported for 54/65 tumors. * One patient was successfully retreated.

One patient was treated for palliative purposes for a large presacral recurrence of endometrial carcinoma (>2000cm³), infiltrating the sacral bone and neural plexus causing severe pain.23 IRE was performed in two sessions. There was only mild paresis resolving spontaneously (grade 1) and no sensory loss or impaired bladder function occurred. Opiate medication was withdrawn. Eight weeks later tumor volume had reduced to 791cm³.

Efficacy

In three studies follow-up after hepatic IRE was not reported or was less than 3 months.30-32 So, 106 patients with 185 liver tumors were analyzed for efficacy: 27 HCC, 56 CRLM, 23 other malignancies (table 3). No deaths due to disease progression were reported. Median tumor size varied from 1.0-3.0cm (range 0.5-8.8cm). Median follow-up period ranged from 3 to 18 months. Primary technique effectiveness varied from 67-100%, secondary technique effectiveness was 55-93% (some tumors were successfully retreated). Several authors reported an increased recurrence risk for larger tumors:23-26,28 Cheung et al achieved 93% ablation success for tumors < 3cm, and 100% for tumors < 2cm at 18 months (p=0.003)(24) and Cannon et al reported 98% efficacy for tumors < 3cm at 12 months.28 Silk et al described local tumor recurrence in five of nine patients, with a median tumor size of 3.0cm.26 Notably, 44% of the tumors treated by Kingham et al were located less than 0.5cm from a major portal vein and 14% were located 0.6-1cm from a major portal vein, which implied a relative
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contra-indication for RFA due to the probability of heat-sink induced recurrence. Technique effectiveness at 6 months was 93%. Similarly, IRE near the right portal vein (n=2) and the middle hepatic vein (n=1) was successful for 2 of 3 HCCs treated by Cheung et al. The tumor that showed residual disease measured 6.1cm.

Martin et al performed open IRE with concurrent surgical procedures for locally advanced pancreatic carcinoma in 54 patients, in combination with chemoradiation. Results were compared to a matched patient group receiving chemoradiation only. Improved LPFS (14 vs. 6 months, p=0.01), DPFS (15 vs. 9 months, p=0.02), and overall survival (20 vs. 13 months, p=0.03) were demonstrated in the IRE-group. In case of progressive disease, most patients had distant progression. After a follow-up of approximately 20 months, no difference between the groups remained due to rapid progression of distant disease. Additionally, median pain score dropped from 5/10 to 3/10 (p=0.04), with a reduction of overall narcotic use (p=0.03). Quality-of-life scores were not registered. Narayanan et al treated three patients with metastatic disease at the time of IRE, all of whom died due to progressive metastatic disease after 3, 4 and 9 months. Of the patients with locally advanced disease, local progression occurred at 1 and 7 months (2/7) and distant progression at 4 months (1/7). Two patients underwent successful resection of the ablated lesion at 4 and 5 months and did not show evidence of disease at last follow-up after 11 and 14 months respectively. The remaining two patients had stable disease at 4 and 6 months. Results are shown in table 4.

Thomson et al performed IRE of 11 renal tumors in 8 patients (7 RCC, 4 other tumors). Median tumor size was 2.7cm (1.6-5.3). Primary technique effectiveness was 45% (5/11). Complete response was only noted for RCC.

Table 4: Efficacy of pancreatic IRE

<table>
<thead>
<tr>
<th>Author</th>
<th>No. patients</th>
<th>Size (cm, median, range)</th>
<th>Approach</th>
<th>FU (median, months)</th>
<th>Stable disease</th>
<th>Local progression</th>
<th>Distant progression</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martin</td>
<td>34</td>
<td>3.2 (1-5.5)</td>
<td>Open (52) Lap (2)</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td>20.2 months (median)</td>
</tr>
<tr>
<td>Bagli</td>
<td>1</td>
<td>4.1</td>
<td>Perc</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Narayanan</td>
<td>7 LapC</td>
<td>3.3 (2.3-7.0)</td>
<td>Perc</td>
<td>8.5 (4-10)</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>6 months: 70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 metastatic</td>
<td></td>
<td></td>
<td>3, 4 and 9 months</td>
</tr>
</tbody>
</table>

Perc, percutaneous; lap, laparoscopic; LapC, locally advanced pancreatic carcinoma. See table 1; ‡ 4/14 patients were excluded for efficacy analysis due to follow-up <3 months.

Two studies reported on IRE of 8 pulmonary tumors in 6 patients from different origin. Median tumor size was 2.6cm (2.0-8.4). After a follow-up of 3 to 12 months, all patients had progressive disease, of which one patient had died.

Discussion

Intentional cell death induced by IRE for tumor ablation has only been studied in the past few years. The potential advantage of preservation of extracellular matrix structures, in the absence of thermal coagulation had undergone extensive preclinical testing before it was introduced in the clinical setting. The results found in this review are further discussed below.

Safety
The electric fields applied in IRE can cause cardiac arrhythmias. Synchronized pulsing with the heart rhythm reduces this risk. This is confirmed by our results: with cardiac gating, only minor arrhythmias occurred with a total incidence of 2% (4/194). Based on this, we expect that with adequate synchronization the risk for severe arrhythmias in a patient without pre-existing cardiac abnormalities, is minimal. Moreover, uncontrolled muscle contractions were prevented with adequate muscle relaxants.

Hepatic IRE was associated with an overall complication rate of 16% (21/129). Numerous liver capsule punctures did not cause subcapsular hemorrhage, and pain appeared similar to pain after RFA. As a comparison, in a review on hepatic RFA in 3670 patients, overall complication rate was 9%, with rates of 7, 10, 10 and 32% for respectively percutaneous, laparoscopic, simple open and combined open RFA. IRE was mostly performed on tumors near or around portal pedicles and impaired patency of these structures caused by IRE occurred in 6/129 cases (5%). Although IRE is believed to be primarily non-thermal, heat development immediately adjacent to the electrodes has been described, which may have caused thermal coagulation and subsequent occlusion of the bile duct that was in direct contact with one of the needles. As a safety precaution, placement of electrodes <2mm to central bile ducts, pancreatic ducts or intestines should thus be avoided. Overall, vascular and biliary structures were mostly preserved, which suggests that IRE may be a safer option than thermal ablation in this area. Further studies with longer follow-up are still needed to confirm these results.

The aim of pancreatic ablation is cytoreduction, leading to better symptom palliation, improved quality-of-life and prolonged survival. To this end, pancreatic RFA was previously investigated. However, due to the organ’s delicate nature and vulnerability to thermal injury, RFA proved unsuitable, with a high complication rate (28-40%) and mortality rate (7.5%). With proven efficacy, a complication rate around 19% for IRE might be acceptable, although reported rates vary widely between studies. In favor of invasive treatment, Martin et al demonstrated comparable morbidity for patients with and without IRE after 4 months, with longer survival and better palliation in the IRE-group.

**Efficacy**

For patients with unresectable hepatic tumors also unsuitable for thermal ablation due to difficult location, chemotherapy with palliative intent is generally the treatment of choice. A new curative treatment option for these patients is therefore of great importance. IRE was used as 'last resort' in most patients. Thus, a primary technique effectiveness of 67-100% (and even higher for smaller tumors), is by all means promising, especially since these unresectable tumors can have a less favorable biological behavior than resectable tumors. Of note, tumors near large vessels did not appear to recur more frequently, which could suggest that treatment effect is indeed not impeded by heat-sink. Considering the limited quality of the data, larger prospective studies are needed to confirm these observations.

Current treatment of locally advanced pancreatic carcinoma consists of chemotherapy (usually gemcitabine or folfirinox), with or without radiotherapy. This leads to a modest increase in survival, often at the expense of severe side-effects. Two studies suggested a treatment benefit for IRE in terms of pain reduction and survival. Due to low patient number and combination of IRE with surgery, it is difficult to determine whether this benefit
Chapter 1.2

is indeed substantial. Future prospective trials should therefore aim to establish the outcome of IRE as stand-alone therapy, including quality-of-life registration.

Due to scarcity of data, no definitive conclusions can be drawn with respect to renal and pulmonary IRE.

Limitations
New cancer treatments are typically best defined from phase III randomized trials comparing the new therapy with the current standard. However, in the field of local tumor ablation this has proven difficult: since its introduction decades ago the number of randomized trials remains very limited. Currently, literature on the clinical application of IRE is scarce with no randomized controlled trials. The majority of our data was extracted from case series and case reports with level 4 evidence and are subject to several limitations: (1) possible publication bias of included retrospective studies and case reports, (2) the presented studies are without controls, low in patient number, and heterogeneous in study design and patient selection, (3) the retrospective design and short follow-up period of some studies may have led to underreporting and missing of (late) complications, (4) several studies combined efficacy results for different tumor types and sizes within one organ, or percutaneous and (combined) surgical procedures, and (5) duration of follow-up and imaging modalities varied across studies. These limitations precluded a meaningful quantitative meta-analysis, other than providing percentages of pooled measures across studies. These pooled measures may incorrectly assume homogeneity between studies. We recognize these limitations and therefore our findings should be regarded with caution. This review presents a snapshot in time in evaluation of IRE and both its content and message are subject to changes based on future science that may be generated in this field. Nevertheless, despite these limitations, the results of IRE with respect to safety and early efficacy found in this review appear encouraging.

Future directions
Several factors that may affect treatment outcome have been identified. Overall, local recurrences were encountered more often after electroporation of larger tumors. A hypothetical solution would be to increase either the number of probes required to treat larger lesions, or the number of probe-repositionings. For example, a four-probe array with an inter-probe distance of 2 cm creates a 3 cm ablation zone. Considering a 1 cm tumor-free margin, this would imply a maximum lesion size of 1 cm for this four-probe array. Misplacement of the probes by a margin of millimeters can result in residual tumor, so accurate intraprocedural imaging is essential. Presumably, precise placement of larger probe arrays is more difficult, especially since probe placement traversing through vulnerable structures should be avoided.

The feasibility of real-time monitoring of hepatic IRE has been demonstrated in animal studies. On ultrasound (US), the ablation zone immediately appears as a hypoechoic area with well-demarcated margins; contrast-enhanced computed tomography (ceCT) shows a well-defined hypodense area, best visible on the portal venous phase. The ablation zone on both imaging modalities correlates well with the pathologically defined zone of cell death. However, results of real-time monitoring have been insufficiently related to oncologic outcome in humans. Follow-up studies should therefore focus on immediate and late imaging characteristics, related to oncologic outcome. Specific per-procedural imaging guidelines
could reassure the interventional radiologist when complete tumor ablation has occurred, thereby increasing treatment efficacy.

During electroporation, cell membrane permeabilization leads to an increase in tissue conductivity and depends on strength, number and duration of the pulses.\textsuperscript{36,31} Animal studies have shown that the changes in electrical conductivity of the ablated tissue – amongst others – determine ablation success. These changes could provide real-time feedback on treatment outcome.\textsuperscript{31-33} However, organ-specific and tumor-specific electric field dose-response studies are lacking, and much remains unknown about the clinical possibilities to destroy malignant tissues with irregular geometries and heterogeneous properties. Knowledge of the electrical and thermal properties of different tissue types would allow for the identification of an optimal electric field, strong enough for maximized tissue ablation, but weak enough to avoid excessive thermal effects.\textsuperscript{34}

As interventional oncologic therapies evolve, they are combined with other treatments such as transarterial chemo-embolization (TACE) and transarterial radio-embolization (TARE) to increase treatment effect. Similarly, a margin of reversibly electroporated tissue exists between the ablation zone and normal tissue directly after IRE. During this temporary permeability of the cell membranes, drugs like chemotherapeutics can travel freely into the cells within this zone, a process known as electrochemotherapy. Capitalizing on this largely unexplored principle, there may be a therapeutic advantage if IRE were combined with systemic chemotherapy to eradicate marginal remnant viable tumour cells.\textsuperscript{35}

**Conclusion**

This systematic review gives an extensive overview of the available evidence of the use of IRE for control of tumors near vulnerable structures such as blood vessels and bile ducts. With only case reports and case series the level of evidence of the available studies is low. Despite these limitations, the results suggest safe use of IRE, which is in accordance with results of pre-clinical studies. Early efficacy on smaller hepatic tumors near vascular structures and portal triads is promising, with reported ablation success reaching 90%, but rapidly decreasing with increasing tumor size. For unresectable pancreatic carcinoma, improved survival and significant pain reduction for IRE combined with radiochemotherapy is suggested, as compared to radiochemotherapy alone.

At this time, it appears that IRE is most suitable for tumors <3cm in diameter that are not eligible for resection or thermal ablation. While much research remains to be done, our results illustrate that the future of IRE appears promising. Alongside the other available local ablation techniques, IRE may become an important tool in the multimodality treatment of cancer in the near future.
Chapter 1.2

Reference list


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Chapter 1.2


“Shock me like an electric eel,
turn me on with your electric feel

MGMT, Electric feel
Chapter 2
Preclinical studies
2.1

Thermal energy during irreversible electroporation and the influence of different ablation parameters

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* contributed equally

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Abstract

Purpose
Irreversible electroporation (IRE) uses high-voltage electric fields to achieve cell death. Although the mechanism of IRE is mainly designated as nonthermal, development of secondary Joule heating is inevitable. The study purpose was to gain understanding of temperature development and distribution during IRE.

Materials and Methods
IRE was performed in a transparent polyacrylamide gel resembling soft tissue. Mechanical effects, changes in temperature gradient, and absolute temperature changes were measured with three different optical techniques (highspeed, color Schlieren, and infrared imaging) to investigate the effect on temperature of variations in voltage, pulse length, active tip length (ATL), interelectrode distance, electrode configuration (parallel, convergent, and divergent), and sequential pulsing (pulse delivery interrupted by breaks). The total delivered energy was calculated.

Results
A temperature gradient, starting at the tips of both electrodes and expanding toward each other, developed immediately with pulse delivery. Temperatures increased with increasing voltage (by 2.5 °C–40.4 °C), pulse length (by 5.3 °C–9.8 °C), ATL (by 5.9 °C–17.6 °C), and interelectrode distance (by 7.6 °C–21.5 °C), in accordance with higher energy delivery. Nonparallel electrode placement resulted in heterogeneous temperature distribution with the peak temperature focused in the area with the shortest interelectrode distance. Sequential pulse delivery significantly reduced the temperature increase compared with continuous pulsing (4.3 °C vs 11.7 °C).

Conclusions
Voltage, pulse length, interelectrode distance, ATL, and electrode configuration each have a strong effect on temperature development and distribution during IRE. Sequential pulsing reduces the extent and volume of thermal distribution and may prove beneficial with respect to procedural safety.
Introduction

Irreversible electroporation (IRE) is increasingly used for the ablation of malignancies, particularly tumors located near vulnerable structures such as bile ducts and blood vessels. The technique uses high-voltage microsecond electrical pulses applied through adjustable needle electrodes. The electrical pulses are proposed to destabilize the existing cellular transmembrane potential, leading to the formation of so-called nanopores in the cellular membrane. In theory, because of the resulting increased cell membrane permeability, the cell loses its homeostatic properties, resulting in cell death. Because the IRE mechanism induces cell death by affecting the cellular membrane, cells are killed in a targeted region, without damage to the collagen and other interstitial tissue constituents. Critical structures like major vasculature and ductal systems may therefore be preserved. The sparing of critical structures is the primary characteristic that distinguishes IRE from other local therapies, offering a therapeutic option for targeting tissues that are contra-indicated for surgical resection, thermal ablation, or radiation therapy. In the light of this important benefit, IRE has shown promising results in the ablation of centrally located hepatic, locally advanced pancreatic and prostate tumors.

The formation of nanoscale defects occurs independently of thermally-induced processes, and the non-thermal mechanism was demonstrated in a large soft tissue sarcoma and during an intracranial procedure. Nonetheless, recent studies have shown that the therapeutic application of IRE will result in secondary Joule heating that can induce thermal damage. Given the fact that the underlying rationale for the clinical application paradigms of IRE are in large part based on the assumption of the non-thermal mechanism of cell death, characterization and quantification of the thermal effects of IRE is necessary to ensure safe but effective ablations.

We hypothesize that thermal distribution during IRE ablations varies in respect to the ablation settings depending on the amount of the delivered Joules. The heating effects will initially be observed around the needles and will subsequently merge after substantial energy delivery. Additionally, it was hypothesized that sequential pulsing results in a smaller increase of temperature than consecutive pulse delivery, because of the intermittent cooling periods without Joule delivery. It was furthermore hypothesized that electrolysis of water might occur during IRE pulsing in water-based gel, because by using direct current through an ionic substance, an interchange of ions takes place causing this effect. The primary goals of this study are the visualization of physical effects of IRE pulses, quantification of the development and distribution of thermal energy during IRE using optical approaches and to determine the influence of different ablation parameters on the thermal outcome. The secondary goals of this study are the evaluation of the effect of sequential (pulse delivery interrupted by breaks) versus consecutive pulse delivery and the evaluation of non-parallel electrode placement on the thermal effect during IRE. The further identification of the thermal component of IRE is vital for the improved safety of IRE in interventional oncology.

Materials and methods

Tissue mimicking gel phantom

We used a transparent polyacrylamide gel of which the characteristics mimic soft biological...
Chapter 2.1

tissue with respect to mechanical properties, and electrical and thermal conduction. For 100 ml gel we used 60 ml saline (NaCl 0.9%), 50 mg ammonium persulfate, 40 ml 30% acrylamide/bis solution and 80 μl tetramethylethylenediamine. The gels were casted by pouring the liquid material into a fixed mold. Dimensions of the gel were 10 cm width, 8 cm height, and 1.5 cm thickness, which allowed for electrode placement similar to in-vivo settings.

IRE-procedure

The IRE-procedure was performed using the Nanoknife® IRE console (AngioDynamics, Latham, New York). For the standard ablation setting, two monopolar 19-gauge needle electrodes were placed in the gel, exactly parallel using a grid and 5 +/- 1 mm from the gel surface (figure 1A-B). The proximal aspect of the active tip was constantly ~4 cm from the top surface. The default ablation settings were 15 mm interelectrode distance, 15 mm active tip length (ATL), delivering 1×90 pulses with a pulse length of 90 μsec, 90 pulses/minute and pulse intensity of 1000 V/cm. The influence of the following ablation parameters on the temperature was objectified: Voltage (-ranging from 500 to 2500 V; interelectrode distance 10mm), pulse length (50, 70 and 90 μsec), interelectrode distance (ranging from 5 to 25 mm for 1000 V/cm), ATL (ranging from 5 to 25 mm) and electrode orientation (parallel, divergent and convergent). Also, continuous delivery of 120 pulses (2 × 60 with an 18 seconds inevitable pause required for the generator recharge) was compared to sequential pulse delivery (4×30 pulses and 2×60 pulses interrupted by breaks of 30, 60 and 90 seconds), as was the effect of converging and diverging electrodes under a 45° angle.

Figure 1: (A) Close-up image showing thermal camera and inserted electrodes in gel. (B) Schematic image of the electrodes in the gel with standard settings (interelectrode distance of 15 mm and active tip length of 15 mm). (C) Analysis of temperature recordings with three regions of interest.

Physical effects of IRE

To visualize physical phenomena during individual pulses, 10 IRE pulses were registered using a high-speed camera (Photron Fastcam MC2, San Diego, CA, USA) mounted in front of the gel showing a 20 mm diameter close-up image of the electrodes capturing images at a frequency of 50 to 8000 frames per second (time resolution from 20 ms to 125 μs). Temperature gradient measurements

The initial temperature effects during the first pulses (which also reflect the position of the highest currents along the needle surface) were registered using the color Schlieren imaging technique. This technique allows visualization of small changes in optical density, induced
by temperature gradients or local stresses. The degree of deflection of the parallel light rays, caused by the temperature gradient, is color coded by a spatial filter (rainbow filter), resulting in a qualitative pseudo 'thermal' image recorded by the high-speed camera (figure 2) at 250 frames per second (4 ms/frame).

**Figure 2**: Schematic image of color Schlieren imaging setup.

### Absolute temperature measurements

The effect of variation in ablation settings on the absolute temperature and distribution at the gel surface was investigated using a Xenics Gobi-384 thermal camera (Xenics, Leuven, Belgium). This infrared camera uses long wavelengths (8-12 μm range) to record thermal changes of a minimum of 0.05 °C at a resolution of 384 × 288 pixels (25-μm pitch). The camera is calibrated at a range of -20°C to 120°C.

### Analysis and statistics

Reproducibility, by means of average temperature difference and standard deviation over multiple measurements (5x), was tested using default settings. The results of the experiments proved to be consistent within 1 °C, which was used as the standard error within the experiments. After the validation of the investigational setup, the subsequent experiments were performed once to observe general trends between parameters. For each experiment, three ROIs were selected at a minimum of 100 pixels per ROI to follow temperature over time. The ROI with the highest mean temperature at the most intense portion of the image was used to calculate the temperatures. Each individual measurement point in time is acquired with a minimal and maximal temperature per ROI (figure 1C). The difference between this minimal and maximal temperature was used to calculate the percentile error for each individual data point. Herein, the average error for all data points in time was calculated. Because the baseline temperature of the gel varied (ranging from 12 to 16 °C), the relative phantom surface temperature (ΔT) was determined between the start (T0) and maximal measured temperature of each ablation. The change in delivered current (ΔA) as determined by the IRE-console was noted. The resultant total of delivered energy E in Joule was calculated using the following formula:

\[ E = V \times I \times N_p \times t_d \]

Where V is the applied voltage, I is the used current in amps, \( N_p \) = the number of pulses and \( t_d \) is the pulse duration time in seconds.
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Results

Physical effect of IRE

During each pulse a light flash was visible at the negative electrode and small gas bubbles were formed predominantly along the negative electrode (figure 3A). Vapor formation, resulting in cavities in the gel focused in the converged tip of the negative electrode. The cavity enlarged in volume with each pulse, related to the amount of delivered energy per pulse, with a maximum of 2.4 mm (figure 3B).

![Figure 3: High-speed camera images showing (A) Start of electric current with light flash at the negative needle electrode (125 f/s) (B) Vapor formation during the first three consecutive pulses at the negative electrode (1000 f/s). The background shows the light source.](image)

Temperature gradient measurements

The temperature gradient distribution is shown in figure 4A-D during 10 pulses. Figure 4A shows the absence of a gradient before pulsing. The initial gradient reflects the area of the highest current density at the electrode tips (figure 4B), expanding alongside the entire active length. The temperature field builds up with each pulse (figure 4C) and decreases after completion of the last pulse (figure 4D). No discrepancy was observed between the negative

![Figure 4: High-speed color Schlieren images showing the temperature gradient distribution before (A) and during an IRE pulse train of 10 pulses (B-C) and during the subsequent relaxation (D) using default settings (15 mm inter-electrode distance, 15 mm ATL, delivering 1×90 pulses with a pulse length of 90 μsec, 90 pulses/minute and pulse intensity of 1000 V/cm).](image)
Thermal energy during IRE
and the positive electrode.

**Absolute temperatures measurements**

*Figure 5* shows the thermal images of the maximum temperatures measured at the gel surface for each voltage. A regular increase in $\Delta T$ was observed with higher voltages. After each IRE-ablation, a rise in current ($\Delta A$) was detected resembling the current increase reported during clinical IRE procedures. Similar to the temperature effect, the rise in amperage was positively correlated to the total amount of energy delivered in Joule. A similar effect was seen for pulse length (*figure 6*), ATL (*figure 7*), and interelectrode distance (*figure 8*). The resulting graphs and tables showing the regular temperature increase followed by a decrease (cooling time), when the pulse delivery was completed, are included in the figures and marked with an asterisk.

<table>
<thead>
<tr>
<th>Voltage (V/cm)</th>
<th>Energy (J)</th>
<th>$\Delta A$ (A)</th>
<th>$T_{\text{max}}$ (°C)</th>
<th>$\Delta T$ (°C)</th>
<th>Error on $\Delta T$ (%)</th>
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<tr>
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<td>20</td>
<td>41.1</td>
<td>30.1</td>
<td>±2</td>
</tr>
<tr>
<td>2500</td>
<td>810</td>
<td>17</td>
<td>55.7</td>
<td>40.4</td>
<td>±2</td>
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</tbody>
</table>

*Figure 5*: Temperature (T) and current development (A) over time for various voltages and resultant dissipated energy. * Represents the end of pulse delivery.
Chapter 2.1

**Figure 6:** Temperature ($T$) and current development ($A$) over time for various pulse lengths and resultant dissipated energy. * Represents the end of pulse delivery.

<table>
<thead>
<tr>
<th>Pulse length [µs]</th>
<th>Energy [J]</th>
<th>$\Delta I$ [A]</th>
<th>$T_{\text{max}}$ [°C]</th>
<th>$\Delta T$</th>
<th>Error on $\Delta T$ [%]</th>
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<tr>
<td>50</td>
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<td>3</td>
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<td>±4</td>
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<td>4</td>
<td>20.4</td>
<td>9.8</td>
<td>±3</td>
</tr>
</tbody>
</table>

**Figure 7:** Temperature distribution and temperature ($T$) and current development ($A$) over time for various active tip lengths and resultant total dissipated energy. * Represents the end of pulse delivery.

<table>
<thead>
<tr>
<th>Active tip length [mm]</th>
<th>Energy [J]</th>
<th>$\Delta I$ [A]</th>
<th>$T_{\text{max}}$ [°C]</th>
<th>$\Delta T$</th>
<th>Error on $\Delta T$ [%]</th>
</tr>
</thead>
<tbody>
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<td>28.2</td>
<td>16.3</td>
<td>±3</td>
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</tbody>
</table>
Thermal energy during IRE

Figure 8: Temperature (T) and current development (A) over time for various inter-electrode distances with 1,000 V/cm and resultant total dissipated energy. Asterisk represents the end of pulse delivery.
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In figure 9, all temperature measurements are plotted against the total delivered energy for a constant interelectrode distance (15 mm). In first order approximation, the energy $[E]$ is dissipated in a constant volume $[V]$ around the electrode resulting in a temperature increase with the linear relation: $\Delta T = \frac{E}{(V \times 4.2 \text{ J})}$ (in which 4.2 J represents the amount of energy required to heat up 1 cm$^3$ of water with 1 °C). The volume $V = 4.7 \text{ cm}^3$ is derived from the linear fitting. This volume is in good accordance assuming a cylindrical volume heated around each electrode with a diameter of 13 mm and a length of 15 mm (2 x 2.3 cm$^3$).

Continuous pulsing with 120 pulses resulted in a rise of almost 12 °C. However, when pulses were delivered sequentially, the temperature decreased during each break, resulting in a significant reduction of the $\Delta T$. Furthermore, by extending the pause between the pulse series, the resultant $\Delta T$ was lower. The table shows the amount of dissipated energy, which is constant for each ablation (figure 10).

With converging as well as with diverging electrodes in a 45° angle, the temperature focused in the area where the electrodes were closest to each other (5 mm). The total temperature increase in this focal point was higher than the total temperature increase for exact parallel electrode placement (figure 11).

The thermal camera measures temperatures at the gel surface at 5 mm distance from the electrodes. Therefore, the actual absolute temperature at the electrode surface is higher than displayed. Extrapolation of the temperatures at 3, 5, 10, 15 and 20 mm from the electrodes results in an exponential curve, which illustrates that the temperature closer to the electrodes is much higher, and quickly decreases further away from the electrodes (figure 12).
Thermal energy during IRE

Figure 10: Continuous pulsing versus sequential pulsing. A+E: Continuous pulsing ablation (2 × 60 pulses in black; indentation caused by required generator recharge following 60 consecutive pulses.) B-D: Sequential pulsing ablations of 4 × 30 pulses with breaks of 30 (green), 60 (blue) and 90 (red) seconds. F-H: Ablations of 6 × 20 pulses with breaks of 30 (green), 60 (blue), and 90 (red) seconds.

Figure 11: The temperature distribution during (A) parallel, (B) divergent, and (C) convergent electrode placement (5-mm interelectrode distance).
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Discussion

Recently, the nomenclature regarding IRE as a non-thermal ablation technique was extensively refuted.\textsuperscript{3,11,15} While cell-scale IRE effects are well described to provide a non-thermal method for cell death,\textsuperscript{16–18} there is substantial evidence that pulse protocols of IRE in the clinical setting induce thermal damage within the ablation zone.\textsuperscript{2, 7, 8, 12} Thermal damage is a function of temperature and exposure duration, and care must be taken to ensure that the cumulative effects do not induce damage to the heat-susceptible structures in the vicinity of the ablated region that could lead to complications.\textsuperscript{3,4} To the best of our knowledge the exact distribution of thermal energy around the electrodes during ablation has not been visualized before. Our data confirm that IRE causes a substantial temperature increase (59.7 °Celsius).

Gas formation

The development of gas bubbles from the start of the first pulse accompanied by the flash at the tip of the negative electrode, as visualized by the high-speed camera, indicates very high local temperatures. This results in explosive vaporization of water at the electrode tip followed by a rebound implosion due to subsequent condensation, temporarily creating a small cavity in the gel. Subsequently, due to the electric current passing through the saline-based gel, it is likely that an electrolytic process (decomposition of water [H\textsubscript{2}O] into oxygen [O\textsubscript{2}] and hydrogen gas [H\textsubscript{2}]) occurs. Both effects probably contribute to the formation of the bubbles.

Distribution and geometry of thermal effects

Although the first order approximation of the dissipated energy reliably predicts the temperature increase, an exponential temperature gradient exists between the electrode (i.e. the thermal source) and the surrounding environment,\textsuperscript{19} as shown in figure 12. Extrapolation to the actual electrode surface suggests a substantially higher local temperature. Furthermore, when electrodes are placed asymmetrically, the thermal effect focuses in the area with the shortest interelectrode distance. Given the time-temperature relationship for heat-induced killing following the Arrhenius equation,\textsuperscript{20} a temperature increase up to 10 °C for several
minutes seems acceptable. For these reasons we recommend a minimum electrode distance of 5mm (figure 12) from critical structures and even greater near the focus of the active tips for angulated pairs. Furthermore, since increasing interelectrode distance (at constant \( V/cm \)) generates higher local temperatures, we advise to keep the interelectrode distance rather small (i.e. 10-15 mm), to better control the temperature when IRE is performed near critical structures.

**Pulse sequences**

Sequential pulsing with pulse trains of 20 or 30 pulses significantly reduced the temperature increase, depending on the duration of the breaks. Aside from the fact that lower temperatures improve procedural safety, Appelbaum et al recently showed that multiple shorter cycles of energy application using a four-probe array created larger ablation zones, at the cost of increased treatment duration. The authors hypothesized that the increase in electrical conductivity induced by an IRE pulse persists after the initial pulse. The shifts of cellular contents such as solutes, caused by the opening of IRE-induced pores in the cellular membrane, occur in the order of minutes rather than seconds. So, besides the number of pulses at a given voltage alone, timing might also influence ablation zone volume. To this extent, IRE may behave similarly to cryoablation where freeze-thaw cycles have been shown to increase the zone of cell death. In the clinical setting, 3-6 electrodes are generally used for ablation, resulting in 3-11 electrode pairs. If trains of e.g. 20 pulses are applied to each of the electrode pairs in a repetitive, cyclical fashion, this will automatically result in a long pause between pulse-sets for individual electrode pairs, so no additional pause would need to be incorporated, so procedure duration can be controlled.

**Conductivity**

Interestingly, similar to ablation of in situ human tissue, we too noted a steady rise in amperage during each pulse ablation cycle. In the used acellular gel, this is clearly not ascribed to increased cell membrane permeability. A possible explanation for the increase in conductivity (and decrease in resistance) of the gel during electroporation is the rise in temperature of the gel, implying a conductivity increase with increasing temperature. If this assumption is correct, one could argue that the decrease in tissue resistance during clinical IRE may not (solely) be attributed to increased cell membrane permeability, but may be caused by the increased tissue temperature. Measuring changes in electrical properties of cells has been proposed for determining the effectiveness of electroporation protocols in individual cells and in cell cultures. Furthermore, Dunki-Jacobs et al proposed that the decrease in impedance should be used during IRE, and suggested a required current increase of approximately 12-15 amperes for successful ablation, with repetition of the protocol in case of a lower increase. But our study proved that caution should be taken when repeating the electroporation protocol to achieve the desired current rise, since the cumulated energy may cause thermal damage.

**Limitations**

This study has several limitations. Because the electrical and thermal properties of the gel may differ from normal tissue – e.g. the gel was colder and not perfused – the temperature curves cannot be directly translated into the clinical setting. In-vivo ablations will be influenced by tissue-specific thermal and electrical conductivity resulting in altered temperature distribution. The results of this study should therefore be interpreted as describing important
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trends only. Analysis of the peaks of the temperature curves occasionally showed an inaccurate registration of the start of the ablation since the maximum temperatures were not always synchronously displayed. Despite these limitations, the obtained temperature and current changes seem to be comparable with in-vivo measurements.\textsuperscript{10,11} Although we used a grid to place the electrodes exactly parallel with the desired interelectrode distance aiming at a precise 5 mm distance to the gel wall, a small displacement of ±1.0 mm may have occurred incidentally, which may have resulted in higher or lower temperatures measured at the gel surface of a few degrees. This might explain the discrepancy we measured for 10 mm interelectrode distance, which resulted in a higher temperature increase compared to 15 mm. Furthermore, because of the short distance of the electrodes to the anterior surface (5 mm), the surface temperature we measured may have been higher than in the clinical setting, since the gel-air boundary possibly limits heat dispersion. Nevertheless, the rise in temperature had a consistent positive correlation with the total amount of Joule energy delivered. An additional limitation is that after the investigational setup had been validated, the experiments were performed once, which limits the possibility of statistical analysis. Furthermore, the true dependence of the temperature development and distribution on interelectrode distance cannot be evidently determined, since the amount of energy was partially defined by the used voltages. Last, it is not known whether the performed ablations would have led to actual and complete cell death of the electroporated tissue in the oncoligic setting. To determine this, the experiments should be performed in an in-vivo setting.

The thermal component of IRE surrounding the electrodes, can no longer be ignored and every physician should take this into account when planning and performing clinical IRE. Specifically, in clinical practice even higher voltages are used than in the present study. Researchers are now faced with the challenging task to develop optimal treatment algorithms that are strong enough to create complete cell death, but weak enough to avoid thermal damage in areas where this can have detrimental effects. Nonetheless, it is conceivable that the thermal element of IRE may have a synergistic effect on treatment efficacy. For example, higher temperatures may increase the size of the actual ablation zone. Also, thermal ablation has been suggested to trigger the release of pro-inflammatory, anti-cancer mediators, which activates the adaptive immune system and elicits an anti-tumor immune response against residual viable cancer cells in the treatment area as well as distal, non-treated cancer cells.\textsuperscript{27-29} This concept of a local therapy having a systemic response - the "abscopal effect" - has also been suggested for IRE.\textsuperscript{30,31} Maybe a synergistic effect of thermal and electrical cell destruction will induce the greatest anti-tumor effect. The immunologic effect of thermal ablation and IRE is the current focus of several trials.

Conclusion

In conclusion, during IRE ablations, varying voltage, pulse length, interelectrode distance, active length exposure and electrode configuration all have a significant effect on the temperature development in good correlation with the dissipated energy near the electrodes. To this extent, sequential pulsing reduces the extent and volume of thermal damage and may prove beneficial with respect to procedural safety. In order to ensure complete ablation whilst preventing thermal damage, the oncoligic efficacy with the different ablation settings – especially the protocol for sequential pulsing - should be validated in animal and clinical studies.
References


Chapter 2.1


Thermal energy during IRE
2.2

The influence of a metal stent on the distribution of thermal energy of thermal energy during irreversible electroporation

Hester J Scheffer, Jantien A Vogel, Willemien van den Bos, Krijn P van Lienden, Marc GH Besselink, Martin JC van Gemert, Cees WM van der Geld, Martijn R Meijerink, John H Klaessens, Rudolf M Verdaasdonk

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**Abstract**

**Purpose**

Irreversible electroporation (IRE) uses short duration, high-voltage electrical pulses to induce cell death via nanoscale defects resulting from altered transmembrane potential. The technique is gaining interest for ablations in unresectable pancreatic and hepatobiliary cancer. Metal stents are often used for palliative biliary drainage in these patients, but are currently seen as an absolute contraindication for IRE due to the perceived risk of direct heating of the metal and its surroundings. This study investigates the thermal and tissue viability changes due to a metal stent during IRE.

**Methods**

IRE was performed in a homogeneous tissue model (polyacrylamide gel), without and with a metal stent placed perpendicular and parallel to the electrodes, delivering 90 and 270 pulses (15-35 A, 90 μsec, 1.5 cm active tip exposure, 1.5 cm interelectrode distance, 1000-1500 V/cm, 90 pulses/min), and in-vivo in a porcine liver (4 ablations). Temperature changes were measured with an infrared thermal camera and with fiber-optic probes. Tissue viability after in-vivo IRE was investigated macroscopically using 5-triphenyltetrazolium chloride (TTC) vitality staining.

**Results**

In the gel, direct stent-heating was not observed. Contrarily, the presence of a stent between the electrodes caused a higher increase in median temperature near the electrodes (23.2 vs 13.3 °C [90 pulses]; p = 0.021, and 33.1 vs 24.8 °C [270 pulses]; p = 0.242). In-vivo, no temperature difference was observed for ablations with and without a stent. Tissue examination showed white coagulation 1mm around the electrodes only. A rim of vital tissue remained around the stent, whereas ablation without stent resulted in complete tissue avitality.

**Conclusion**

IRE in the vicinity of a metal stent does not cause notable direct heating of the metal, but results in higher temperatures around the electrodes and remnant viable tissue. Future studies should determine for which clinical indications IRE in the presence of metal stents is safe and effective.
Introduction

Irreversible electroporation (IRE) is a relatively novel ablation modality that uses electrical energy to induce cell death.\textsuperscript{1} Electrodes are placed around a tumor, through which high-voltage, but sub-millisecond electrical pulses are applied at a low frequency (0.5–2 Hz). As opposed to thermal ablation techniques, the electrical pulses are designed to distort the pre-existing cellular membrane potential, leading to disruption of the lipid bilayer, after which the cell loses its homeostatic properties and dies.\textsuperscript{6–8} Preclinical studies have shown that within the ablation zone IRE mostly affects cells, leaving the supporting extracellular matrix structures relatively intact.\textsuperscript{6–9} This preservation of gross anatomic architecture allows tumors near vascular and biliary structures that are otherwise unresectable or unamenable to thermal-based modalities, to be ablated safely.\textsuperscript{9,10}

Although IRE was initially introduced as being non-thermal, several studies have now demonstrated that clinical therapies employing high pulse numbers over the electrode pairs (70–200 per pair) in an electric conductive medium inevitably produces cumulative secondary heat due to Joule heating that may affect treatments\textsuperscript{11–15} especially in the immediate vicinity of the electrodes where the current density is highest.\textsuperscript{12,16} Because IRE is typically used around structures vulnerable to thermal injury, the search for optimal ablation settings minimizing the probability for thermal damage whilst still achieving complete tumor cell death, continues.\textsuperscript{16,17}

Early clinical application of IRE in hepatopancreatobiliary tumors has raised the question whether thermal injury occurs in the presence of a metal stent, since these patients frequently present with a bare metal Wallstent in situ to resolve obstructive jaundice caused by tumor compression on the common bile duct. These metal stents have a smaller risk of migration, occlusion, therapeutic failure, and cholangitis compared to plastic biliary endoprosthesis,\textsuperscript{18} but can only be removed with extensive surgical or endoscopic manipulation. Given the high electrical and thermal conductivity of metal relative to mammalian tissue, the safety and efficacy to perform IRE in the vicinity of a metal stent has been subject to debate.\textsuperscript{14,19,20} The manufacturer of the Nanoknife\textsuperscript{\textregistered} electroporation device has stated that the presence of a metal stent within the ablation zone is an absolute contraindication. As a result, many patients with pancreatic or extrahepatic cholangiocarcinoma with a metal stent are withheld IRE treatment.

Recently, a fatal case was published in which several complications following IRE ablation in the pancreatic head region with a metal stent in situ were described, including perforation of the duodenum and transverse colon in close proximity to the stent, and bleeding from a branch of the superior mesenteric artery.\textsuperscript{19} In a reply to the published case, we used a mathematical model to calculate the potential effect of a metal stent on heat development, which seemed negligible.\textsuperscript{21} Oppositely, a second case was recently published in which IRE was performed successfully around a metal stent for perihilar cholangiocarcinoma.\textsuperscript{22}

Still, the range and extent of effects of a metal stent within the ablation zone is unknown and requires additional controlled experimental evaluation. Given the great impact of an absolute contraindication, a precise evaluation of the effect of IRE around metal objects is warranted. The purpose of this study was to determine the distribution of thermal energy and the potential clinical implication of IRE around a metal stent using experimental models.
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Materials and methods

In-vitro experiment

Self-expandable nickel and titanium (nitinol) stents (Epic, Boston Scientific, Marlborough, Massachusetts, US) with a 5 mm diameter, 60 mm length and 0.19 mm mesh thickness were placed inside a transparent gel made of 150 ml saline (NaCl 0.9%), 125 mg ammonium persulfate, 100 ml 30% acrylamide/bis solution and 200 μl tetramethylethyleendiamine, mimicking human soft tissue with respect to electrical and thermal conduction properties. One electrode was placed on each side of and parallel to the metal stent (‘stent-IRE’), with an inter-electrode distance (IED) of 1.5 cm, active tip length of 1.5 cm, and 0.5 cm distance to the tissue surface. The same setup was used without a stent between the electrodes (‘no-stent-IRE’) (figure 1). For ablation, the NanoKnife® IRE console (AngioDynamics, Latham, New York, US) was set at 1x90 and 3x90 (270) pulses, with a pulse length of 90 μsec, 90 pulses/minute and a pulse intensity of 1500 V (1000 V/cm voltage-to-distance ratio), aiming at a delivered current of 15–35 Amperes (A). Each experiment was repeated five times. The temperature of the tissue surface was visualized using a Xenics Gobi-384 thermal camera, which records thermal changes of 0.05 °C. Temperature data were extracted using the Xeneth software package (Xenet, Leuven, Belgium).

![Figure 1: Setup of IRE ablations performed in a tissue phantom. (A) electrodes parallel to stent; (B) electrodes perpendicular to stent; (C) electrodes without stent.](image)

In-vivo experiment

Following an approved Institutional Animal Ethics Committee protocol, four IRE ablations were performed in the liver periphery of a domestic farm pig weighing approximately 50 kg, with and without stent, with two ablations each for 90 and 270 pulses. The animal was sedated with intramuscular ketamine (10–15 mg/kg), midazolam (1–1.5 mg/kg), and atropine (1.5 ml/50 kg). After intubation, anesthesia was maintained through inhaled isoflurane (2%-4%) and intravenous ketamine (2 mg/kg/h), sufentanil (5–10 mg/kg/h), midazolam (1–2 mg/kg/h), and rocuronium (2–2.5 mg/kg/h). Before IRE, intravenous bolus injections of rocuronium (1–1.5 mg/kg) were administered for complete muscle relaxation. The animal was placed in the supine position and the liver was exposed through a medial laparotomy.
IRE with a metal stent

Stents were placed through a puncture hole and expanded to 0.5 cm diameter parallel to and 0.5 cm beneath the liver surface using ultrasound guidance. Electrodes were positioned parallel to the stent at a distance of 0.5 cm on either side of the stent, corresponding to an IED of 1.5 cm. To measure the temperature within the ablated area, two fiber-optic temperature probes, with a 1 mm diameter (TRUE Lumiterm X5, Ipitek, Carlsbad, CA, US) were placed directly against either side of the stent at an equal depth to the IRE electrode tips, registering 0.05°C temperature differences (figure 2). The same experimental setup for no-stent-IRE was used. The liver surface temperature was measured using the thermal camera mounted to the operating table. Ablations were performed using the same settings as with the in-vitro experiments except for the voltage, which was 2250 V (1500 V/cm voltage-to-distance ratio). During electroporation the animal was kept in apnea. Thirty minutes after the last ablation the animal was euthanized by exsanguination. Tissue evaluation consisted of macroscopic examination. Each specimen was sliced into two parts, either parallel or perpendicular to the electrodes. Of each specimen, one half was immediately fixated in formalin. The other half was incubated with 5-triphenyltetrazolium chloride (TTC) vitality staining for 30 minutes at 37 °C prior to formalin fixation to identify areas of irreversible cell damage.25,26

Analysis and statistics

The delivered energy per pulse in Joule (J) was calculated using the following formula:

\[ \text{Energy (J)} = \text{Voltage (V)} \times \text{Current at first pulse (A)} \times \text{pulse duration (s)} \]

Continuous variables were presented as median and range. Non-parametric tests were used for non-normally distributed data, where a p-value of <0.05 was considered statistically significant. Data were analyzed using SPSS version 20.0 (IBM statistics, Inc., Chicago).

Figure 2: Setup of IRE ablations performed in in-vivo porcine liver showing the electrodes (brown/gray) and temperature probes (blue). No-stent-IRE (A, cross-sectional; B, longitudinal) and stent-IRE (C, cross-sectional; D, longitudinal). Green arrow represents the distance to the liver surface.
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Results

In-vitro experiment

During all in-vitro ablations (stent-IRE and no-stent-IRE), a constant temperature rise was detected, which peaked at the electrode tips and gradually decayed to the area between and then away from the electrodes. The highest increase in temperature was always measured at the tip of the electrodes. Temperature increase was larger after 270 pulses than after 90 pulses. Median temperature increases for the different ablation protocols are shown in Table 1; median currents reached are shown in Table 2. Figure 3 shows the representative results of the thermal camera during 90 pulses no-stent-IRE. For stent-IRE, no direct stent-heating was observed (Figure 4 and 5). The maximum temperature increase at the location of the stent in stent-IRE was similar to the same region in no-stent-IRE (p = 0.592 [90 pulses] and p = 0.567 [270 pulses]), but was reached approximately 10-20 seconds later. The maximum temperature increase measured at the tip of the electrodes in stent-IRE was higher than in no-stent-IRE (p = 0.021 for 90 pulses and p = 0.242 for 270 pulses, figure 4 and 5). Median current at the first pulse of IRE was higher in stent-IRE (p=0.044, Table 2) as well as current rise, but this difference was not significant (p = 0.266 [90 pulses] and p = 1.000 [270 pulses], Table 3).

Table 1. Absolute maximum temperature increase measured between the electrodes and at the tip of the electrodes.

<table>
<thead>
<tr>
<th>Median maximum increase in temperature in °C (range) in tissue phantom</th>
<th>No stent</th>
<th>Stent parallel</th>
<th>Stent perpendicular</th>
</tr>
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<tbody>
<tr>
<td>Between electrodes</td>
<td>90 pulses</td>
<td>10.2 (4.7-12.6)</td>
<td>8.6 (6.9-11.5)*</td>
</tr>
<tr>
<td></td>
<td>270 pulses</td>
<td>23.6 (17.0-26.7)</td>
<td>21.0 (19.7-23.5)**</td>
</tr>
<tr>
<td>Electrode tip</td>
<td>90 pulses</td>
<td>13.3 (11.6-14.1)</td>
<td>19.4 (14.7-21.3)*</td>
</tr>
<tr>
<td></td>
<td>270 pulses</td>
<td>24.8 (17.2-26.6)</td>
<td>26.5 (25.5-35.4)**</td>
</tr>
</tbody>
</table>

Data represent the median and range of 5 experiments. * 4 experiments, 1 aborted due to high current, ** 3 experiments, 2 aborted due to high current.

Table 2. Median current at the first pulse of each ablation in tissue phantom (range)

<table>
<thead>
<tr>
<th>Median current at first pulse in Amperes (range)</th>
<th>Energy dissipation in Joule/pulse (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-stent-IRE</td>
<td>17 (14-18)</td>
</tr>
<tr>
<td>Stent-IRE</td>
<td>20 (16-22)</td>
</tr>
<tr>
<td>Mann-Whitney U test (two-sided)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Table 3. Current increase during in vitro IRE.

<table>
<thead>
<tr>
<th>Median current increase in Amperes (range)</th>
<th>No-stent-IRE</th>
<th>Stent-IRE</th>
<th>Mann-Whitney U</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 pulses</td>
<td>5 (4-7)</td>
<td>6.5 (5-11)</td>
<td>0.266</td>
</tr>
<tr>
<td>270 pulses</td>
<td>10 (8-13)</td>
<td>13 (11-18)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
IRE with a metal stent

In-vivo experiments

Temperatures increased during all ablations (table 4, figure 6 and 7). In no-stent-IRE, gross pathology showed a homogeneous appearing ablation zone, continuous from one electrode to the other (figure 8A). Accordingly, TTC vitality staining demonstrated complete avitality of the ablated area around and in between the electrodes visible as a TTC-negative, unstained area (figure 8C), compared to the vital TTC-positive (red stained) liver tissue distant from the ablation zone. Stent-IRE resulted in an inhomogeneous ablation zone (figure 8B and D) with an area of viable liver tissue immediately surrounding the stent. No coagulative necrosis was
noted in the area adjacent to the stent. White coagulation, representing coagulative necrosis caused by thermal damage, was only observed in the immediate vicinity of the electrodes, in both stent-IRE and no-stent-IRE.

**Figure 5:** Stent-IRE, perpendicular. Thermal camera images during 90 pulses (A-D). (E) Graph showing the temperature increase at the surface of the gel, 5 mm from (I) the active tip of the electrode, (II) inside the stent and (III) at the margin of the stent. (A) pre-IRE (B) after 60 IRE pulses (C) after 90 IRE pulses (D) 60 sec after the last IRE pulse.

**Figure 6:** No-stent-IRE. Thermal effects during 270 pulses in porcine liver; Thermal camera showing the temperature increase at the surface of the liver (5 mm from the active tip of the electrode) (A) before and (B) directly after 270 pulses (I: surface above ablation zone, II: surface of normal liver). (C) Increase in surface temperature measured with fiber-optic probes during ablation. (D) Increase in temperature measured with thermal camera during ablation (the disturbance at 100 sec is caused by an ultrasound measurement).
Discussion

It was never disputed that every electric field, including a field for irreversible electroporation, produces a thermal effect. Yet, from 2005 onwards, scientists showed that IRE could be isolated from thermal effects and used by itself to produce substantial volumes of tissue ablation in vivo, with negligible thermal effects. The application of IRE for the therapeutic ablation of tumors however, has evolved to use more aggressive energy regimens, with higher voltage and higher pulse number protocols. These high-energy regimens have shown to generate potentially harmful thermal effects, which is reaffirmed in the present study. Much effort should therefore be put in the development of clinical pulse protocols that mitigate these thermal effects and maintain IRE as the vastly predominant modality of tissue death.

Due to the relative infancy of clinical IRE and the presumably heterogeneous energy distribution resulting from metallic stents in the treatment region, major concerns have been raised about the use of IRE in proximity to metal stents. Chiefly, the presumed heating of the stent and its surroundings as a result of its high electrical conductivity are a concern. This study demonstrated that the temperature of the stent itself does not exceed the temperature...
Chapter 2.2

of the adjacent tissue during IRE, implying that there is no direct heating of the stent and that the absolute contraindication in this respect is unsubstantiated. This corresponds to our previous calculations.\(^2\) On the other hand, two different effects were detected which warrant further exploration and consideration in regard to their influence on IRE outcomes: 1) a higher temperature increase around the electrodes and 2) a remnant viable rim immediately surrounding the stent.

A higher temperature around the electrodes is important when considering the location of electrode placement. Usually electrodes are placed within and around the tumor, including vital tissue where vulnerable structures such as bile ducts, nerves, and non-tumorous vessels traverse. Due to the increased heat development around the electrodes, the risk of damage to these structures is increased. This stresses the essence of calculative and precise electrode placement.

The remaining rim of vital liver tissue immediately surrounding the stent is disconcerting, since it may negatively influence oncological outcome. This concern especially relates to tumors in direct contact with the stent - such as perihilar cholangiocarcinomas and other liver tumors. IRE may still be of value when treatment is mainly based on palliation, since

\[\text{Figure 8: Gross pathology without staining (A, B, D right specimen) and with TTC vitality staining (C, D left specimen) of IRE ablated liver; (A, B) 90 pulses, sliced perpendicular to electrode placement, (A) no-stent-IRE and (B) stent-IRE, (C, D) 270 pulses, sliced parallel to electrode placement, (C) no-stent-IRE and (D) stent-IRE. White arrowheads represent the location of needle placement; white arrows represent the center of the ablation zone.}\]
tumor debulking may prolong stent patency, thereby reducing disease-related morbidity and postpone tumor progression. From a different perspective, the vital rim could also be considered advantageous. In cases where the stent is placed in a non-invaded bile duct, the 1 mm vital rim can be considered additional protection of the damage-susceptible bile duct. For each individual case of IRE around a stent, the possibilities and limitations should therefore be deliberated cautiously.

In the fatal case published by Månsson et al., the causality between the stent and the complications could not be established. While this serious complication is indisputably alarming, our results show that direct heating of the stent should not have been the cause. However, the increased temperature surrounding the electrodes may have contributed to the development of the complications, especially if one of the electrodes was placed near the duodenal wall or a large vessel. Neal and colleagues showed no difference in electrical behavior between ablations with and without symmetrically arranged expired radiotherapy seeds in a non-animal model as well as in ex- and in-vivo canine prostate. Similarly, further in-silico evaluations predicted no significant alteration of the electric field and temperature development. As opposed to the presented study, the tissue adjacent to the seeds within the realm of ablation had the same appearance as the ablated prostate tissue without seeds. The authors stated that larger implants like stents might exert a larger effect on current distribution around the electrodes, which would accord with our results. Grounded metal plates near or within the ablation area can result in large regional changes in electric field distribution, by pulling the electric field away from the positive electrode, shown by Ben David et al. However, this pull will not occur with electrically isolated metal objects, like stents. Compared to our findings, Dunki-Jacobs et al measured a larger maximum temperature difference of 18 °C at 0.5 cm distance from the electrodes, between IRE with and without stent and metal clips placed deep in the porcine liver. However, details about number of electrodes and pulses used, and whether the results referred to stents or clips were not provided. Remarkably, an ineffective ablation was reported with a clip, suggesting a significantly changed electric field distribution. Although this could correlate with our findings, the exact details were not provided.

Our study has several limitations. First, our in vitro absolute temperature measurements should be interpreted as describing important trends only and are not representative for the temperatures achieved in perfused tissue, because 1. the camera measures the surface temperature rather than the exact stent, tissue or electrode temperatures, 2. living tissue has a higher baseline temperature and will therefore result in a smaller temperature gradient, and 3. living tissue is perfused and will conduct temperatures faster and further away from the ablation zone compared to the gel, which may explain why we observed smaller temperature changes in vivo. Another limitation is the interval from ablation to tissue harvesting, which may have been too short to allow completion of IRE cell death processes. Since the specimens were harvested approximately 2-3 hours after the ablation, the effect of cell death may not have been maximally present at the time of evaluation and our macroscopic findings may have been an underestimation of the actual zone of cell death. Furthermore, we did not account for the physical effect of metal stents in (often infected) biliary obstruction or cancerous tissue, which alters the cellular and stromal tissue aspects, and introduces uncertainties regarding the electrical properties. Finally, the animal experiments were only performed four times and should therefore be interpreted with caution.
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The assumption that the metal of the stent would be directly heated in the electrical field of the electrodes is improbable since metal is a good thermal and electrical conductor. However, it can be expected that the metal stent will highly distort the distribution of the electrical field and the pathway of the current between the electrodes - performing similarly to a Faraday's cage - leading the current around rather than through the stent.

The higher temperature increase around the electrodes can be explained by the lower net resistance in the area of the stent. Given a constant voltage, the net current will increase as a result of decreasing resistance. Indeed, our measurements showed a 15% higher current at the first pulse in stent-IRE compared to no-stent-IRE ($p = 0.044$). Consequently, more energy is deposited ($P = V \times I = P \times R$), resulting in a higher temperature increase around the electrodes.

In light of the fact that the actual mechanisms of cell death from IRE remain a topic of discussion by some regarding the potential role of thermal coagulation in the ablation process, multiple hypotheses for the rim of viable tissue around the stent can be surmised. One way or the other, the metal stent will distort both the electrical and thermal field distribution, resulting in an unpredictable ablation zone that apparently results in a small rim of vital tissue near the stent. This could either be ascribed to a less effective IRE effect due to the distorted electrical field, or to a heat-sink effect of the stent if thermal effects would have a substantial role in the mechanism of action. Future experiments should provide more insight in the contribution of both mechanisms.

Current literature on the influence of metal objects remains limited and future work should further characterize the effects. We are currently preparing an animal study in which the findings from this work will be verified and further analyzed. In the meantime, we advise that whenever possible, placement of an uncovered Wallstent should be avoided and a retrievable plastic endoprosthesis or covered endoscopically retrievable Wallstent should be placed instead. Also, in open procedures the stent should be removed peroperatively prior to IRE.31 Still, for patients in which the stent cannot be removed or in which removal imposes a significant risk, IRE may be considered.

**Conclusion**

IRE in the vicinity of a metal stent does not cause notable increased heating of the metal stent, but results in higher temperatures around the electrodes. In vivo, a remnant viable tissue region immediately adjacent to the stent was observed. These findings reinforce the appeal to either place plastic biliary endoprostheses or to remove metal stents prior to IRE whenever possible. Future studies should determine for which clinical indications IRE in the presence of metal stents is safe and effective.
References


IRE with a metal stent
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30. Davalos R V, Bhonsle S, Neal RE. Implications and considerations of thermal effects when applying irreversible electroporation tissue ablation therapy. Prostate. 2015;1118:1114–1118. doi:10.1002/pro.22986

IRE with a metal stent
“There is no pain, you are receding
a distant ship, smoke on the horizon
You are only coming through in waves
The dream is gone
I have become comfortably numb

Pink Floyd, Comfortably numb
Chapter 3

Anesthetic management
3.1

Anaesthetic management for open and percutaneous irreversible electroporation

Hester J Scheffer*, Karin Nielsen*, Jenny M Vieveen, Aukje AJM van Tilborg, Sybren Meijer, Cornelis van Kuijk, Petrousjka (MP) van den Tol, Martijn R Meijerink, Arthur (RA) Bouwman

*contributed equally

British Journal of Anaesthesia 2014;113(6)985-92
Abstract

Background
Irreversible electroporation (IRE) is a novel tumour ablation technique involving repetitive application of electrical energy around a tumour. The use of pulsed electrical gradients carries a risk of cardiac arrhythmias, severe muscle contractions and seizures. We aimed to identify IRE-related risks and the appropriate precautions for anaesthetic management.

Methods
All patients that were treated with IRE were prospectively included. Exclusion criteria were arrhythmias, congestive heart failure, active coronary artery disease and epilepsy. All procedures were performed under general anaesthesia with complete muscle relaxation during ECG-synchronized pulsing. Adverse events, cardiovascular effects, blood samples, cerebral activity and postprocedural pain were analysed.

Results
Twenty-eight patients underwent thirty IRE sessions for tumours in the liver, pancreas, kidney and lesser pelvis. No major adverse events occurred during IRE. Median systolic and diastolic blood pressure increased by 44 mmHg (range -7–108 mmHg) and 19 mmHg (range 1–50 mmHg) respectively. Two transient minor cardiac arrhythmias without haemodynamic consequences were observed. Muscle contractions were mild and IRE caused no reactive brain activity on a simplified EEG. Pain in the first 24-hours after percutaneous IRE was generally mild, but higher pain scores were reported after pancreatic treatment (mean VAS-score 3; range 0–9).

Conclusions
Side-effects during IRE on tumours in liver, pancreas, kidney and lesser pelvis seem mild and manageable when current recommendations for anaesthesia management, including deep muscle relaxation and ECG synchronised pulsing, are followed. Electrical pulses do not seem to cause reactive cerebral activity and evidence for pre-existing atrial fibrillation as an absolute contra-indication for IRE is questionable.
Anaesthetic management for IRE

Introduction

Irreversible electroporation (IRE) is a novel tumour ablation technique based on the local application of an electrical field between two or more electrodes inserted around a tumour. Multiple cycles of short, extremely high-voltage electrical pulses alter the transmembrane potential of tumour cells, leading to the creation of nanoscale defects in the lipid bilayer of the cell membrane, increasing membrane permeability. With the appropriate electrical parameters (90 pulses of 70 μsec; electric field strength 1500 V/cm; delivered current 20-50 Ampère), the membrane permeability becomes permanent and the cell eventually dies due to loss of homeostasis.1,2 Because cell death in IRE is based on electrical energy rather than thermal energy, the technique has two advantages over thermal ablation techniques like radiofrequency ablation (RFA). Firstly, whilst IRE effectively destroys all cells within the ablation area, the extracellular matrix is preserved. As a consequence, vascular, biliary and nervous structures rich in extracellular collagenous and elastic structures remain intact.3,4 Secondly, IRE is unaffected by the so-called 'heat-sink effect', in which incomplete tumour ablation may occur near large vessels due to loss of heat via blood flow.5 Therefore, IRE may represent an effective alternative for tumours that cannot be resected or thermally ablated due to unfavourable location. In light of the promising results of the first trials investigating the safety and efficacy of IRE in different organs, we anticipate that the therapeutic use of IRE will expand rapidly in the near future.6-9

For the anaesthesiologist, the pulsatile application of electrical pulses with a very high voltage presents specific challenges, including the possible triggering of cardiac arrhythmias caused by increased cell membrane permeability of electroporated tissue, which opens a path for ion transport.10 Also, severe muscle contractions and epileptic seizures could occur due to stimulation of muscular or nervous tissue.11 Therefore, specific precautions in intraprocedural management are required in order to safely perform IRE. For example, as complete muscle paralysis is necessary to prevent muscle contractions, all IRE procedures require general anaesthesia and the use of muscle relaxants. Furthermore, to prevent arrhythmias the electrical pulses must be administered synchronously to the heart rhythm, since external electrical stimuli delivered during the absolute refractory period of the heart are incapable of inducing an action potential.10

Current literature on anaesthetic management for IRE procedures is limited to a single publication by Ball and colleagues, who reported their initial experience in 21 patients treated with CT-guided percutaneous IRE for hepatic, renal and pulmonary tumours and formulated guidelines for this procedure.11 In our study, we aimed to broaden the experience of open and percutaneous IRE to an additional patient population with different tumour types and to confirm previous formulated guidelines for anaesthetic management. To this end, we specifically focused on IRE-related side-effects and the appropriate precautions with respect to anaesthetic management of open and percutaneous procedures in different organs.

Methods

This study was conducted with the approval of the Medical Ethics Committee of the VU University Medical Center. The study was designed and conducted in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki. All patients provided written informed consent.
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**Patients**

All patients treated with open or percutaneous IRE between August 2012 and September 2013 were prospectively included in a database and analysed. This included patients participating in the COLDFIRE-I trial, in which patients who were already scheduled for surgical resection of colorectal liver metastases (CRLM) were treated with IRE during surgery 60 minutes prior to resection (Clinicaltrials.gov registration number: NCT01799044). All other patients underwent IRE in the liver, pancreas, kidney or lesser pelvis on clinical indication, due to proximity of the tumour to vital structures precluding surgical resection or thermal ablation. A designated multidisciplinary board determined local treatment. All patients had a histologically proven malignancy and underwent appropriate preprocedural imaging. Inclusion criteria were ASA classification ≤3 and adequate bone marrow, hepatic and renal function. Exclusion criteria were cardiac arrhythmias requiring anti-arrhythmic therapy or pacemaker/implantable cardioverter-defibrillator, a history of congestive heart failure (NYHA-class >2), active coronary artery disease, uncontrolled hypertension and epilepsy.

**Anaesthetic management**

Preoperative screening was performed with specific emphasis on contraindications for IRE. Patients undergoing laparotomy received a thoracic epidural prior to surgery. The anaesthesia technique used was standardized to avoid bias. Based on personal preferences as well as a practical issue (not all anaesthesia machines in the radiology department of our institution are equipped with proper scavenging systems that allow the use of volatile anesthetics), total intravenous anaesthesia was induced with propofol (2 mg.kg⁻¹), sufentanil (0.3 mcg.kg⁻¹) and rocuronium (0.6 mg.kg⁻¹) and maintained with propofol and remifentanil.

The Accusync ECG gating device (model 72; Milford, Connecticut) was connected to a 5-lead ECG to allow IRE pulses to be synchronized with the refractory period of the heart to avoid arrhythmias. Two defibrillation pads were placed and connected to a defibrillator as a precautionary measure. Immediately prior to IRE, complete muscle relaxation was confirmed by a train-of-four (TOF)-ratio of 0, using a peripheral nerve stimulator (TOF-Watch®, MIPM, Mammendorfer, Germany) to assess neuromuscular transmission. When necessary, an additional dose of rocuronium was administered.

After laparotomy, the epidural stayed in situ for at least 3 days. Pain control after percutaneous procedures was managed with acetaminophen combined with an NSAID (diclofenac), and an opioid (piritramide) if needed.

**Safety analysis**

All adverse events were graded according to the Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0). Cardiac rhythm, blood pressure and saturation were continuously monitored. ECGs were monitored continuously until discharge from the recovery ward, and another 12-lead ECG was made one day post-IRE. Blood samples were evaluated with a special emphasis on serum electrolytes, renal function and hepatic or pancreatic enzymes that could identify biochemical disturbances possibly caused by cellular destruction. These samples were drawn within 7 days prior to IRE, within 5 minutes following IRE and at least one day after the procedure. To monitor brain activity and the effect of pulses on the cerebrum prior to and during IRE, a simplified electroencephalogram (EEG) was made in six patients using the Thymatron System IV (Schwind Benelux Medical Electronics BV, Oosterbeek, The Netherlands).
Anaesthetic management for IRE

Netherlands). Postprocedural pain was scored three times a day during hospital admission, using the Visual Analogue Scale (VAS).

**Intervention**

All procedures were performed by a board-certified interventional radiologist trained in IRE. Before IRE, the size and shape of the target lesion, including a 1 cm tumour-free margin, were defined; this determined the number and configuration of the electrodes. Two or more insulated 15 cm needle electrodes with an exposure length of 2 cm (liver, kidney and lesser pelvis) or 1.5 cm (pancreas) were placed in the outer border of the tumour with an interprobe distance of 2.0 cm (± 0.2 cm). During laparotomy, intraoperative ultrasound (Alpha7, Hitachi Aloka Medical, Ltd. Tokyo, Japan) was used to aid electrode placement. For percutaneous procedures, the electrodes were advanced under CT fluoroscopy (Volume Zoom, Siemens, Erlangen, Germany), with or without ultrasound (figure 1). To allow needle placement under CT fluoroscopy, ventilation was briefly stopped.

After confirmation of correct electrode position, the appropriate parameters for voltage (1500 V/cm), number of pulses (90) and pulse interval (70 μs) were set, and ablation was started using the NanoKnife, a low-energy direct-current ablation device (AngioDynamics, Latham, NY). The pulses were synchronized with the heart rhythm, as detailed above. The delivered current should lie between 20-50 A, and in case of overcurrent (>50 A) a safety mechanism automatically turns off energy delivery to prevent thermal injury. If necessary, electrodes were repositioned for renewed ablation delivery until the ablation zone fully covered the tumour.

**Statistical analysis**

All data were described and analysed; continuous variables were presented as mean and standard deviation (normal distribution), median and range (non-normal distribution), or frequencies and percentages (categorical variables). A p-value <0.05 was considered significant. Analysis was performed using SPSS 20.0 (SPSS Inc, Chicago).

**Results**

A total of 28 patients with ASA-classification I (n=4), II (n=23) and III (n=1) were treated with IRE in 30 sessions. Thirteen patients were treated during laparotomy for CRLM, of whom 10 patients participated in the COLDFIRE study. All these patients underwent resection...
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and RFA of additional lesions. The remaining 15 patients were all treated percutaneously for CRLM (n=5), pancreatic carcinoma (n=5), cholangiocarcinoma (n=1), hepatic adenoma (n=1), renal cell carcinoma (n=1) and presacral metastasis of colorectal carcinoma (n=2). Two patients were treated twice due to local recurrence (presacral metastasis and cholangiocarcinoma). Two to six electrodes were used for each procedure, depending on the size of the target lesion. Median ablation time, defined as the interval between the first and the last pulse was 14 minutes (range 4-120) and was determined by lesion size and the need for needle repositionings for overlapping ablations. Median total procedure time was 206 minutes (range 90-315) and median length of hospital admission was 8 days following laparotomy (range 5-12 days) and 4 days following percutaneous IRE (range 2-20). Patient and procedure characteristics are provided in table 1.

Cardiovascular effects: hypertension and arrhythmias

Table 1: Patient and IRE procedure characteristics

<table>
<thead>
<tr>
<th></th>
<th>Liver (open)</th>
<th>Liver (perc)</th>
<th>Pancreas</th>
<th>Kidney</th>
<th>Sacrum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Number of lesions</td>
<td>19</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Number of IRE procedures</td>
<td>1±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Median tumor size (mm) (range)</td>
<td>20 (5-53)</td>
<td>28 (14-50)</td>
<td>40 (33-50)</td>
<td>32</td>
<td>48 (34-50)</td>
<td>28 (5-53)</td>
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<tr>
<td>Muscle movement</td>
<td>−</td>
<td>±/−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HD parameters (n, median and range)</td>
<td>Arthymias</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rise SBP (mmHg)</td>
<td>29 (7 – 54)</td>
<td>45 (10-80)</td>
<td>60 (30-108)</td>
<td>60</td>
<td>50 (42-50)</td>
<td>44 (7-108)</td>
</tr>
<tr>
<td>Rise DBP (mmHg)</td>
<td>16 (1-23)</td>
<td>30 (5-50)</td>
<td>30 (25-38)</td>
<td>40</td>
<td>25 (18-28)</td>
<td>19 (1-50)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>2 (7-20)</td>
<td>6 (0-32)</td>
<td>18 (10-22)</td>
<td>2</td>
<td>10 (0-15)</td>
<td>10 (7 – 32)</td>
</tr>
<tr>
<td>Maximum VAS (median, range)</td>
<td>4 (0-18)</td>
<td>3 (0-8)</td>
<td>4 (2-9)</td>
<td>0</td>
<td>2 (0-3)</td>
<td>3 (0-9)</td>
</tr>
</tbody>
</table>

HD, haemodynamics; VAS, visual analogue score; SBP, systolic blood pressure; DBP, diastolic blood pressure; bpm, beats per minute; perc, percutaneous.

During electroporation, an elevation of systolic and diastolic blood pressure was observed in most patients, with a median of 44 mmHg (range -7-108 mmHg) and 19 mmHg (range 1-50 mmHg) compared to baseline. This was most pronounced during pancreatic IRE (60 mmHg and 30 mmHg). This elevation was easily managed with additional propofol and remifentanil, and blood pressure returned to baseline within minutes after IRE. Heart rate showed a moderate increase during electroporation (median 10/min, range -7-32), and seemed more profound during IRE of the pancreas (18/min) than of the other organs (8/min), although this difference was not significant.

During two IRE procedures, a minor self-limiting cardiac arrhythmia was observed. Ventricular extrasystole was observed during open IRE apical in the liver near the left diaphragm. Cardiac rhythm normalized after abortion of the procedure and could be continued after removal of the electrode that was closest to the heart. The second arrhythmia, bigeminy with premature ventricular complex, occurred during pancreatic ablation but disappeared within 5 minutes of the end of the procedure. Neither minor arrhythmia led to haemodynamic instability. All ECGs made one day after IRE were without abnormalities and similar to the ECG made prior to the intervention.
**Muscle contractions during IRE**

Muscle relaxation was verified before the start of IRE in all patients, expressed as a TOF=0. While generalized contractions of skeletal muscles were successfully prevented with rocuronium, mild contractions confined to the treatment area were still visible. During open IRE, this was noticed with minimal pulsatile movement of the electrodes. Local contractions were more profound during percutaneous procedures, especially when electrodes were inserted through large muscles to achieve optimal positioning. Isolated contractions of the gluteus maximus and the rectus abdominus muscle were remarkable during IRE in the lesser pelvis (dorsal approach) and in the pancreas, respectively. The muscle contractions never resulted in dislocation of the electrodes. Median duration between the last pulse and termination of the procedure was 20 minutes for percutaneous (range 3-89) and 113 minutes for open procedures (range 32-172). In 11/15 percutaneous procedures a median dosage of 400mg sugammadex (range 200-1600 mg) and in 6/13 open procedures 200 mg was used to reverse neuromuscular blockade.

**Laboratory values**

Significant electrolyte abnormalities were not observed in any patient. Serum pH was measured during open procedures, but no disturbances were noted. Renal function remained unremarkable during IRE and in the postoperative period, except for a clinically insignificant decline in glomerular filtration rate in the patient who was treated in the kidney (69 to 59 ml/min/1.73 m²). Further analysis revealed elevation of hepatic and pancreatic enzymes in all patients directly after IRE of the liver and pancreas respectively. This was most pronounced on the first postoperative day and decreased thereafter. One patient developed a pancreatitis with bile leakage, characterized by a persistent increase in pancreatic enzymes, pain and fever. These values normalized after antibiotic treatment and drainage. The postoperative elevation of transaminases in the 13 patients that underwent additional RFA and liver segment resection (COLDFIRE study) could not be solely attributed to IRE, since these additional procedures are commonly associated with such increases.

**Cerebral monitoring**

Prior to induction of general anaesthesia, normal cerebral activity was registered. After induction, brain activity became minimal in five (5/6) patients. During IRE, each electrical pulse was clearly registered as an artefact in all six patients (figure 2). Importantly, no reactive (epileptic) activity was observed.

**Complications**

Apart from the previously mentioned minor arrhythmias, no adverse events occurred during the procedures. In the postoperative period after open IRE, five complications occurred in four patients (4/13), two of which were major (re-laparotomy due to alleged persisting haemorrhage; postoperative pain). None of these complications were considered directly related to IRE but most likely to the surgical procedure.

Postprocedural complications after percutaneous IRE also occurred in five patients (5/17) after hepatic, pancreatic, renal and lesser pelvic tumour ablation (table 2). Of these, two were considered major complications (CTCAE grade ≥ 3).
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Postoperative pain

Because all patients who underwent open IRE of the liver also had additional resection or thermal ablation, postoperative pain was unlikely to be solely related to IRE. In all but one patient, pain was well-managed with thoracic epidural analgesia.

Postprocedural pain after percutaneous IRE in the liver, pancreas, kidney and lesser pelvis was mild, with a mean maximum reported VAS score of 3 during hospital admission (range 0-9).

Of these sites, pain following pancreatic IRE was the most severe with a mean maximum VAS of 4 (range 2-9) (table 2).

Table 2: Complications during and after IRE

<table>
<thead>
<tr>
<th>Treatment site</th>
<th>Complication</th>
<th>N</th>
<th>Grade</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (open)</td>
<td>Arhythmia</td>
<td>1</td>
<td>I</td>
<td>Removal of one electrode</td>
</tr>
<tr>
<td></td>
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<td>III</td>
<td>Re-laparotomy</td>
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<tr>
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<tr>
<td>Pelvis</td>
<td>Nerve function loss</td>
<td>1</td>
<td>II/III</td>
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</table>
Discussion

IRE is a promising new technique for the local treatment of tumours ineligible for surgical resection or thermal ablation. The local application of an extremely high voltage presents several challenges in anaesthetic management that need to be anticipated.\textsuperscript{10,11} Results of the present study indicate that adverse effects may include cardiac arrhythmias, a transient rise in blood pressure and muscle contractions. However, these adverse effects were generally mild and easily managed. Although the size of our population precludes definitive conclusions, our results are in concordance with previous observations that IRE appears safe and feasible when a dedicated anaesthetic team takes the proper precautions.

Strong electrical currents are known to have the potential to cause arrhythmias, including ventricular tachycardia or even ventricular fibrillation.\textsuperscript{12} Without the use of cardiac synchronization, Ball and colleagues observed brief runs of ventricular tachycardia in seven patients, which seemed to occur more frequently in close proximity to the heart.\textsuperscript{11} Of these, four were associated with a decrease in arterial blood pressure, but immediately after completion of the 10-pulse treatment cycle blood pressure and ECG rhythm returned to normal. These and other ventricular arrhythmias should be prevented by synchronizing IRE pulse delivery with the absolute refractory period of the cardiac cycle (microseconds after the R-wave) using R-wave detection.\textsuperscript{9,10} Since the use of synchronized pulsing, only minor arrhythmias have been reported in the literature, all of which resolved spontaneously after the procedure was aborted.\textsuperscript{7,8,11} In our study, a mild and self-limiting cardiac arrhythmia was observed in two patients, one of which occurred during IRE near the left diaphragm, which is in line with previous results. According to the manufacturer, IRE is contraindicated in patients with pre-existent cardiac arrhythmias, as R-wave detection may be less reliable here, although there is no literature to support this. Since other applications such as electrical cardioversion to treat atrial fibrillation also rely on accurate R-wave detection for synchronized defibrillation shock application, this contraindication may be relative and needs to be further investigated.\textsuperscript{34}

A transient rise in blood pressure during electroporation, usually without a simultaneous rise in heart rate, was observed in all but one patient, confirming previous observations.\textsuperscript{11} In our study, the increase in blood pressure was effectively treated with additional propofol and remifentanil. Although the median values for the changes in blood pressure were moderate, the maximum individual response can be considered dangerous in a patient with a history of cardiovascular disease. The exact mechanism behind this rise is unclear, but stimulation of the autonomous nervous system is a likely explanation. Whether this autonomous stimulation is caused by direct stimulation or by pain perception and how it is best prevented, remains to be clarified before patients with major cardiovascular disease can be treated.

When applying high voltage pulses, neuromuscular blockade is required to avoid uncontrolled severe contractions. Ball and colleagues reported that inadequately paralyzed patients developed contractions of the entire upper body with each pulse, similar to a grand mal seizure.\textsuperscript{11} In our study, even after confirmation of complete medicinal neuromuscular blockade (TOF=0), the electric pulses induced at least mild muscle contractions confined to the treatment area in all percutaneous procedures. These contractions were probably due to leakage current inducing a regional electromagnetic field causing direct muscle depolarization, which is not prevented by non-depolarizing neuromuscular blocking agents.
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Another contraindication for IRE, and noted by the manufacturer, is epilepsy or previous seizure activity, since the electrical discharges could theoretically trigger a seizure through pulsatile brain stimulation. Electrical pulses used in electroconvulsive therapy for severe depression induce seizure activity if a frequency of > 5Hz is reached, but whether IRE can induce seizures has not been firmly established. Therefore, we analysed the effect of IRE on the brain in non-epileptic patients by monitoring cerebral activity with a simplified EEG. We found an absence of background brain activity during IRE in 5/6 patients. This can be attributed to the use of propofol, which is a known suppressor of cerebral activity. The high voltage seen during an electrical pulse was never followed by a reactive cerebral response in any of the patients. Indeed, the depth of anaesthesia may entirely preclude seizures. The likelihood of seizure induction by IRE is also reduced by the synchronization of pulses with the heart rhythm, meaning that they will never reach the minimum frequency of 5Hz required to provoke a seizure. Based on these results and arguments, epileptic seizure risk during IRE is probably very low. If IRE were indicated in a patient with epilepsy, we suggest that use of cerebral monitoring during the procedure would allow quick seizure recognition and treatment initiation.

Postprocedural pain was previously investigated after percutaneous IRE of kidney, lung and liver. Pain was well-managed with oral analgesics in almost all patients, if treatment was necessary at all. Another study showed that postprocedural pain in patients treated with either RFA or IRE for hepatocellular carcinoma was comparable, with no significant difference in analgesic requirements and without the need for epidural analgesia in any of the patients. This was confirmed by our results, which showed that percutaneous IRE of the liver, kidney and lesser pelvis did not cause much discomfort. After percutaneous IRE of the pancreas a previous study found that most patients experienced (CTCAE) grade I pain. Here, pancreatic ablation was associated with the greatest pain in the first 24-hours post-IRE, with maximum VAS scores up to 9, which may be explained by the anatomic location of the pancreas near the coeliac plexus, combined with the induction of a reactive pancreatitis caused by the treatment. This pain was still effectively managed using a multimodal pain treatment consisting of acetaminophen in combination with an NSAID and opioid. The past years the relationship between opioid use and cancer recurrence has been investigated. So far, no significant differences can be identified in most studies between the post-operative use of opioids or other analgesic techniques. These data are extracted from conflicting results from retrospective analyses after resection of primary cancers and not from treatment of metastases. Prospective trials are awaited to draw distinctive conclusions on the role of opioids in cancer recurrence. The relatively mild postoperative pain and limited opioid use after (percutaneous) IRE in most of our patients may be beneficial in this respect, but the numbers treated are too low to make any hard statements. On the other hand, the relatively long duration of the procedure may have confounded the experienced postoperative pain in some patients, since continuous remifentanil infusion is associated with hyperalgesia.

The results in this study represent our first experience with IRE in a relatively small and heterogeneous patient group in terms of target organ and treatment approach, which permitted evaluation of IRE-related anaesthetic challenges in different organs than were reported previously. Overall, perioperative management of the patients was similar. The absence of serious cardiac arrhythmias and muscle contractions supports the assumption that they can be prevented by ECG-synchronized pulsing and profound neuromuscular blockade.
Anaesthetic management for IRE

Epilepsy could be regarded as a relative rather than an absolute contra-indication if IRE were considered the only local treatment option. However, our conclusions should be regarded with care because of the small sample size, which is a limitation of this study. Future studies should focus on long-term follow-up, including long-term adverse events, local control and survival rates. From an anaesthetic perspective, it may be worthwhile to further explore the more generalized physiological consequences of IRE, since IRE is intended to induce only local effects. For example, it would be interesting to investigate the mechanism of the rise in blood pressure during IRE and whether localised muscle contractions caused by leakage currents can be prevented by using a depolarizing neuromuscular blocking agent, like succinylcholine.

In conclusion, our study suggests that adverse effects during IRE of the liver, pancreas, kidney and lesser pelvis are generally mild and easily manageable if specific perioperative precautions are taken. These include general anaesthesia with deep muscle relaxation and ECG-synchronized pulsing.
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References

12. Nikolski VP, Efimov IR. Electroporation of the heart. Europace 2005;7 Suppl 2:146–54
“What kind of magic spell to use
Slime and snails
Or puppy dog tails
Thunder or lightning
Something frightening

David Bowie, Magic Dance
Chapter 4

The Liver
Ablation of colorectal liver metastases by irreversible electroporation: results of the COLDFIRE-I ablate-and-resect study

Hester J Scheffer, Karin Nielsen, Aukje AJM van Tilborg, Jenny M Vieveen, Arthur (RA) Bouwman, Geert Kazemier, Hans WM Niessen, Sybren Meijer, Cornelis van Kuijk, Petrousjka (MP) van den Tol, Martijn R Meijerink

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Abstract

Objectives
Irreversible electroporation (IRE) is a new ablation technique that relies on high-voltage electrical pulses. This clinical study evaluates the pathological response of colorectal liver metastases (CRLM) treated with IRE and the clinical safety and feasibility.

Methods
Ten patients with resectable CRLM were included. During laparotomy, the metastases were treated with IRE and resected 60 minutes later. Safety and feasibility were assessed based on adverse events, laboratory values, technical success and intra-operative ultrasound findings. Tissue response was assessed using triphenyl tetrazolium chloride (TTC) vitality staining and (immuno)histochemical stainings (HE, complement-3d and caspase-3).

Results
Ten lesions with a mean diameter of 2.4 cm were successfully electroporated and resected, on average, 84 minutes later (range 51-153 minutes). One minor transient cardiac arrhythmia occurred during IRE. Ultrasonography showed a sharply demarcated hypoechoic ablation zone around the tumour. TTC showed avitality of all lesions, covering the complete tumour in 8/10 lesions. Although immunohistochemistry proved heterogeneous and difficult to interpret within the tumours, it confirmed irreversible cell damage in the tumour-free margin of all specimens.

Conclusion
This ablate-and-resect study demonstrated avitality caused by IRE of CRLM in humans. Further characterisation of tissue- and tumour-specific electrical properties is warranted to improve ablation protocols for maximized tissue ablation.
IRE for CRLM - the COLDFIRE-I study

Introduction

Image-guided ablation techniques have expanded the therapeutic options for local tumour treatment considerably over the past decades, particularly when surgical options are precluded. This has been of paramount importance in the treatment of colorectal liver metastases (CRLM), since 70-80% of the patients are considered unsuitable for surgical resection due to location, size and number of metastases, or due to comorbidity.

Thermal ablation, and especially radiofrequency ablation (RFA), is currently the most commonly applied technique for curative as well as palliative treatment of CRLM if resection is deemed impossible. Although good results can be achieved, with 5-year survival rates approaching surgical resection in selected cases, the technique has two important limitations. The first is the risk of local recurrence because of incomplete ablation. This risk is higher for tumours located near large blood vessels, since heat can be lost to the flowing blood (the so-called 'heat-sink' effect). The second limitation is the risk of thermal damage to adjacent or inlaying vital structures. This often results in a lack of local treatment options for tumours close to large vessels, major bile ducts or other organs. Recently, a novel ablation technique that addresses these limitations was introduced: irreversible electroproporation (IRE).

Irreversible electroproporation is based on the pulsatile application of an electric field (1000-1500V/cm) between electrodes inserted around the tumour. These electrical pulses alter the existing transmembrane potential, which leads to the creation of 'nano-pores' and disruption of cellular membranes. With sufficient amplitude and pulse duration, the membrane changes are permanent and the cell dies through loss of homeostasis.

Preclinical studies have shown that IRE only affects cells within the ablation zone, leaving the supporting extracellular matrix intact. This preservation of gross anatomic architecture thus allows tumours near vascular and biliary structures to be ablated safely. In addition, the non-thermal mechanism of cell death theoretically means that treatment should not be impeded by heat-sink effects.

The results of the first human studies with IRE are promising. However, due to small patient numbers, heterogeneous inclusion criteria and a relatively short follow-up period, extrapolation to general clinical practice is uncertain. Furthermore, the effects of IRE on human cancer cells and the mechanism of cell death remain poorly understood. The primary aim of this prospective ablate-and-resect study for CRLM in humans was to investigate the pathophysiological effect. Secondary aims were clinical safety and feasibility of IRE.

Material and Methods

The study was approved by the local medical ethics committee. All patients gave written informed consent. Study design and conduct were in accordance with Good Clinical Practice and the principles of the declaration of Helsinki, and the trial was registered at Clincaltrials.gov: NCT01799044.

Patients

Between November 2012 and March 2013, ten consecutive patients with CRLM who met inclusion criteria were prospectively included to undergo open IRE and subsequent resection of the treated lesion in one session. All patients were discussed in our multidisciplinary liver
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tumour board prior to inclusion. Each participant underwent a pre-operative workup within six weeks prior to surgery that included F18-FDG PET-CT and contrast enhanced computed tomography (ceCT) and/or magnetic resonance imaging (MRI), and complete anaesthetic screening. Inclusion criteria were at least one resectable F18-FDG PET avid CRLM smaller than 3.5cm and good performance status (American Society of Anesthesiologists (ASA) 1-2). Patients with additional lesions were also included if all lesions were eligible for local treatment by resection or thermal ablation. Exclusion criteria were cardiac arrhythmias or pacemaker, epilepsy, previous local treatment of the target lesion, and chemotherapy less than six weeks prior to the procedure.

Anaesthetic management
Patients received standard haemodynamic monitoring and a thoracic epidural catheter for intra- and postoperative pain relief. General anaesthesia was induced with propofol, sufentanil and rocuronium and maintenance was achieved with propofol and remifentanil. To prevent pulse-induced arrhythmias, the Accusync ECG-gating device (model 72; Milford, Connecticut) was connected to a 5-lead ECG, which synchronizes pulse delivery within the refractory period of the heart. Just before the start of IRE delivery, complete muscle relaxation was induced with rocuronium to prevent generalized muscle contractions and twitch absence was confirmed using a peripheral nerve stimulation test (TOF-Watch, MIPM, Mammendorf, Germany). A 12-lead ECG was made one day postoperatively. The epidural catheter was left in situ for at least three days.

Intervention
A subcostal incision laparotomy was performed for optimal liver exposure. The resectability and three-dimensional measurements of the target lesion were confirmed with intraoperative ultrasound (IOUS) (Prosound Alpha 7, Hitachi AlokaMedical Ltd., Tokyo, Japan). Size and shape, including a 1cm tumour-free margin, determined the number and configuration of the electrodes. Two or more insulated 15cm needle electrodes with an exposure length of 20mm were either placed in the outer border or just outside of the tumour under US-guidance, aiming at an inter-electrode distance of 20mm (±2mm) between electrode pairs, which is considered the most effective treatment distance at which the created ablation zone has an oval shape. All needles were placed parallel to one another to promote homogeneous energy delivery. The NanoKnife (AngioDynamics, Latham, NY), a low-energy direct-current electroporation device, was used for ablation. Electroporation was performed between all electrode pairs that were separated less than 2.5cm from each other, including diagonal ablations. So, for a four-needle configuration the maximum number of combinations used was six. First, ten test-pulses of 1500V/cm with a duration of 70μs were delivered via each electrode pair, after which the delivered current was verified. The target current lies between 20-50A and voltage settings were manually adjusted in case of over- or undercurrent. Subsequently, 80 remaining pulses were administered to reach a total of 90 pulses per electrode pair. For larger tumours, after ablation of the deepest part of the tumour a 1.5cm pull-back of the electrodes could be performed to ablate the superficial part of the tumour. After IRE, additional lesions were either resected or thermally ablated. At least one hour after ablation, the electroporated lesion was resected and submitted for pathological analysis.

Safety
Adverse events were graded according to NCI CTCAE version 4.0. Perioperative
haemodynamic and cardiac parameters were assessed. To investigate whether cellular destruction leads to biochemical disturbances, parameters including pH, sodium, potassium, renal function and liver enzymes including bilirubin were evaluated prior to IRE, directly after IRE (prior to any other intervention), 1- and 3 days after surgery.

Feasibility
Total ablation time (time from placement of the first electrode to removal of the last), IRE delivery time (actual firing time of the electrodes) and number of electrodes placed per lesion were recorded. Technical success was defined as (1) the ability to successfully deliver all planned pulses and (2) complete lesion coverage on post-procedure IOUS.16

Tissue evaluation
Immediately after resection, the specimen was sectioned in 5mm cross-sectional slices. The slice traversing through the midsection of the lesion (largest tumour diameter for spherical lesions) was incubated with 5-triethylol tetrazolium chloride (TTC) at 37°C. TTC is used to identify vital tissue; mitochondrial enzymes from vital cells transform the initially colorless TTC into a red stain called formazan, whereas non-vital cells remain unstained.11 TTC has been shown to predict the area of irreversible cell damage prior to histological confirmation of cell death.17 Each specimen was sectioned in 4μm paraffin slices and stained with hematoxylin and eosin (HE) for histologic structural evaluation, complement-3d to evaluate cell death, and caspase-3 to evaluate apoptosis.18,19

Results
Patients
Ten patients were initially included. At laparotomy, two patients were unexpectedly excluded; in one patient this was due to a technical failure of the NanoKnife before ablation, in the other patient IOUS revealed progression of liver metastases to the extent that local treatment was no longer considered feasible. Therefore, two additional patients were included. One lesion in each patient was treated according to protocol. All lesions were located in the periphery of the liver. Median lesion size was 2.4cm. Although on pre-operative imaging all lesions were smaller than

<table>
<thead>
<tr>
<th>Table 1: Baseline characteristics</th>
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<tr>
<td>No. patients</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age (mean/range)</td>
</tr>
<tr>
<td>ASA</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>Site original tumour</td>
</tr>
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<td>Rectum</td>
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<tr>
<td>Colon</td>
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</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
<tr>
<td>N0</td>
</tr>
<tr>
<td>N1</td>
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<tr>
<td>N2</td>
</tr>
<tr>
<td>M0</td>
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<tr>
<td>M1</td>
</tr>
<tr>
<td>Systemic chemotherapy</td>
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<tr>
<td>Chemotherapy 6-10 weeks prior to IRE</td>
</tr>
<tr>
<td>No chemotherapy</td>
</tr>
<tr>
<td>Previous portal vein embolization</td>
</tr>
<tr>
<td>Previous thermal ablation of other lesions</td>
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</tr>
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<td>Thermal ablation</td>
</tr>
<tr>
<td>Resection only</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>IRE lesion size (cm) (median/range)</td>
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</table>
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3.5cm, one lesion proved larger on IOUS (5.3cm). Median time between IRE and resection was 84 minutes (range 51-153min). Forty-four additional CRLM were either resected or thermally ablated within the same procedure. Baseline characteristics are summarized in table 1.

**Table 2: Feasibility parameters**

<table>
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<th>Parameter</th>
<th>Value</th>
<th>Description</th>
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<td>Technical success</td>
<td>9/10 (90%)</td>
<td>In one procedure 1 of 5 electrode pairs did not complete 90 pulses due to arrhythmia</td>
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<tr>
<td>Mean total ablation time (min., range)</td>
<td>25 (16-75)</td>
<td>Longest ablation impaired by overcurrent, requiring repeated parameter adjustment</td>
</tr>
<tr>
<td>Mean IRE delivery time (sec., range)</td>
<td>182 (90-270)</td>
<td>Depends on number of electrode pairs, which is determined by lesion size</td>
</tr>
<tr>
<td>Median number of electrodes placed (range)</td>
<td>3 (2-5)</td>
<td>2 electrodes (n=4), 3 electrodes (n=3), 4 electrodes (n=2), 5 electrodes (n=1)</td>
</tr>
<tr>
<td>Number of electrode pull-backs</td>
<td>5 (50%)</td>
<td>For ablation with 2 (n=3), 4 (n=1) and 5 (n=1) electrodes</td>
</tr>
<tr>
<td>Complete lesion coverage on intraoperative ultrasound</td>
<td>10 (100%)</td>
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**Safety and feasibility**

The only IRE-related adverse event was an episode of ventricular extra systoles without haemodynamic changes, which occurred during ablation near the left diaphragm. The arrhythmia ceased immediately following procedure abortion and IRE was resumed without complications after removal of the electrode closest to the diaphragm. Systolic and diastolic blood pressure increased in all patients during pulsing, with a mean of 30mmHg (range 10-54mmHg) and 15mmHg (range 8-23mmHg) respectively. This was modified with additional propofol and remifentanil and blood pressure returned to baseline within minutes after IRE. Postoperatively, serum liver transaminases and lactate dehydrogenase were elevated directly after IRE and on the first postoperative day, followed by a rapid decrease on day three. Analyses of the other laboratory values were unremarkable. All ECGs made 24 hours after IRE were similar to preoperative registration.

Besides the technical failure prior to ablation, IRE was technically successful in 9/10 procedures. Mean total ablation time and mean IRE delivery time were 25 minutes and 182 seconds per lesion. The median number of electrodes placed per lesion was 3 (range 2-5). Real-time monitoring with IOUS showed that a 1-2mm hyperechoic area with gas typically formed around the tip of the electrodes (figure 1C). During and immediately after ablation, the ablation zone was characterized by a clearly demarcated hyperechoic area widely encapsulating the metastasis in all cases (figure 1D). Feasibility parameters are shown in table 2.

**Tissue evaluation**

*Macroscopy – fresh tissue*

The ablated area showed a haemorrhagic red-brown discoloration compared to untreated tissue, which was most pronounced in the zone immediately surrounding the lesion (figure 2A). Traversing larger portal, arterial and venous blood vessels and bile ducts within the ablation zone appeared patent and intact.

*Macroscopy – vitality staining*

Twenty-four hours after TTC vitality staining and formalin fixation of the midsection slice, three different zones were identified: avital tumour, avital ablated parenchyma around the
Therefore, at this point, four individual zones were identified: avital tumour tissue (zone I), avital tumour-free margin (zone II), transitional zone between treated and untreated tissue (zone III), and vital liver parenchyma (zone IV).

Microscopy
Each zone was individually investigated. The morphology of the treated tumour (zone I) was highly heterogeneous between and within the specimens, with variable degrees of epithelial ductal proliferation, central necrosis and intraluminal mucus deposition on HE staining.
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However, in all lesions clusters of extravasated erythrocytes were detected to a greater or lesser extent. Areas with positive and negative response to complement-3d were unevenly distributed throughout the tumour, as was caspase-3 activity (figure 3A-C).

On HE, the tumour-free margin (zone II) immediately adjacent to the tumour was characterized by extensive erythrocyte congestion into and between widened sinusoids and infiltration of neutrophilic granulocytes. Although most hepatocytes had increased cytoplasmic volume, the architecture of the hepatic lobules was preserved. Morphologically vacuolated hepatocytes showed strong cytoplasmic and moderate membranous complement-3d activation. Caspase-3 activity in the cell nucleus was evident in 5/10 patients (figure 3D-F) and in accordance with the TTC results there was a clear demarcation between zone I and zone III on microscopy, in which the typical characteristics of zone II rapidly decreased in intensity except for the neutrophilic granulocyte infiltration (figure 1F-H; figure 3G-I). Untreated liver parenchyma (zone IV) had a normal architecture on HE, absent caspase-3 activity and minimal cytoplasmic complement-3d positivity, without membranous staining (figure 3J-L).

Discussion

Evaluation of ablation success in the clinical setting generally relies on imaging alone. In this ablate-and-resect study we combined intraoperative imaging features with histopathologic specimen analysis after IRE. The ultrasonographic ablation zone in our study fully covered the tumour and a tumour-free margin. This corresponded with our TTC findings indicating avitality of that area in 8 of 9 investigated specimens. Our findings are consistent with a previous study of porcine livers, which demonstrated avitality of the ablation zone within 15 minutes after IRE using TTC.11 The tumour and the initially haemorrhagically infiltrated ablation zone turned pale 24 hours after TTC staining and fixation. The appearance of zone III remains unexplained. After termination of all ongoing biochemical processes by formalin fixation for 24 hours, this region did show mitochondrial enzyme activity. The discoloration four weeks later therefore suggests an unknown chemical difference between untreated liver parenchyma (zone IV) and
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Figure 3: Photomicrograph of zones I-IV at 20 × magnification. Stained with HE (upper), complement-3d (middle) and caspase-3 (lower). (A-C) Ablated tumour (zone I) showing ductal proliferation with central necrosis and clusters of extravasated erythrocytes on HE, variable complement-3d and caspase-3 activity. (D-F) Tumour-free margin (zone II) showing widened sinusoids with massive erythrocyte extravasation on HE, strong cytoplasmic complement-3d activity and nuclear caspase-3 activity. (G-I) Transitional zone (zone III) showing overall normal hepatic parenchyma except for marked granulocyte infiltration on HE, with mild complement-3d and caspase-3 staining. (J-K) Untreated liver parenchyma (zone IV) showing normal morphology on HE, minimal cytoplasmic complement-3d and absent caspase-3 activity.

the transition zone (zone III), rather than avitality. Since the literature describes a margin of reversibly electroporated tissue between treated and untreated tissue in the hours after ablation, zone III might represent this zone of reversible electroporation.

Our macroscopic results were supported by additional (immuno)histochemical analyses. The congestion and widening of the sinusoids suggests that intracellular adhesion of hepatocytes has been impaired due to cell membrane disruption. This vascular congestion causes tissue hypoxia, which may further contribute to tumour cell death. In addition we used complement-3d, which indicates irreversible cell damage through retention at the cell membrane. Our specimens showed strong cytoplasmic complement-3d activity in the tumour-free margin, compared to the other zones, but membranous activity in isolated cells only. Cytoplasmic complement-3d activity can recall local production of complement and doesn’t unequivocally prove cell death. However, in combination with the TTC findings, morphological changes and caspase-3 activity in 5/10 patients, it strongly indicates cell death in the tumour-free margin. These results are reminiscent of previous studies with IRE in porcine liver.

It is known that TTC identifies irreversible cell damage at an earlier stage than histology. This explains why TTC indicated avitality in 8/9 investigated tumours, but microscopic irreversible tumour cell damage caused by IRE was harder to objectify due to unevenly
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distributed complement-3d and caspase-3 activity (zone I). Several factors may attribute to this heterogeneity. First, pre-existing tumour necrosis may have precluded apoptosis from occurring, causing uneven caspase-3 distribution. Second, the ablation-to-resection interval may have been too short to allow completion and histologic identification of cell death which is a drawback of the study protocol. This was also suggested in a study with implanted hepatocellular carcinoma in rats; on microscopy one day post-IRE the treated tumour tissue was still mostly viable with only sporadic necrotic characteristics, while adjacent ablated liver tissue was completely necrotic.24 In the following days the area of tumour necrosis expanded and was complete at day 7. The authors suggested that rather than a direct effect of IRE, cell death over time was caused by ischemia and associated hypoxia, since the viable tumour cells were trapped within the necrotic tissue. This hypothesis is supported by the results from Qin et al, who recently demonstrated that the local electrical heterogeneity of tumour tissue increases the electric field threshold for IRE as compared to normal tissue. As a result, not all cells undergo membrane disruption and live tumour patches can remain within the ablated tissue.25

Although the macroscopically visible vascular and ductal structures in the ablation zone appeared patent and intact, the study design does not allow conclusions to be drawn on the integrity of main bile ducts or hilar blood vessels following IRE for central liver tumours, since resectable CRLM are generally located distant from the portal triad. Nevertheless, current literature reporting the early outcome after IRE for hepatic lesions close to the hilum seems promising; no clinically significant bile duct, portal vein or hepatic artery stenoses or occlusions were noted.12,13 The long-term safety of this aspect of the procedure needs to be established in future clinical trials.

As long as the electric field remains below 1500V/cm IRE should be primarily non-thermal.26 The gas formation surrounding the electrodes (figure 1C) is thought to be caused by the electrolysis of water (H2O) into oxygen (O2) and hydrogen gas (H2) by the electric current passing through the tissue. However, heat-induced evaporation around the electrodes has also been described.26 As a safety precaution we therefore recommend that positioning of electrodes <2mm to central bile ducts, pancreatic ducts or intestines be avoided.

The delivery of powerful electrical pulses to human tissue has the potential to cause fatal cardiac arrhythmias, epileptic seizures and extensive muscle contractions and thus requires specific precautions.27 Cardiac screening and synchronised pulsing, along with complete neuromuscular blockade are therefore essential. When these measures are taken, initial clinical studies have indicated that IRE is safe.13,28 Although our small study group prevented us from making valid statements on safety, only one transient and benign arrhythmia occurred during IRE. In this respect, our results are in concordance with previous reports.

Real-time monitoring provides immediate and reliable feedback on IRE progression.29 In our study, the ablation zone directly appeared as a hypoechoic area with well-demarcated margins. Preclinical studies have described this direct effect to be a good initial indication of the actual ablation zone.16,29 This observation requires further validation in clinical practice, with future studies focusing on the correlation between immediate post-IRE imaging, follow-up imaging and local outcome.

To ensure the long-term success of IRE further efforts are needed to reduce the chance of
live tumour cells remaining within the ablation zone. During electroporation, cell membrane permeabilisation leads to an increase in tissue conductivity. Animal studies have shown that these conductivity changes – among others – determine ablation success, and could therefore also provide real-time feedback on treatment outcome.\textsuperscript{30,31} However, organ- and tumour-specific electric field dose-response studies are scarce, and more research on the electric and thermal properties of malignant tissues with irregular geometries is needed to identify an optimal electric field for maximized tissue ablation. Several other factors that were not addressed in the current study have been identified that may contribute to improved efficacy by changing conductivity through the creation of more stable pores, such as increasing the total number of pulses delivered between a probe pair,\textsuperscript{32} the use of high-frequency electrical pulses to allow more uniform electrical current flow in heterogeneous tissue\textsuperscript{33} and the cyclical deposition of IRE application.\textsuperscript{34} In conclusion, this study demonstrated actual macroscopic avitality of malignant liver tumours in humans as early as one hour after IRE. Although immunohistochemistry showed complete cell death in the tumour-free margin, microscopic cell death in the tumour itself was more difficult to objectify. This may be explained by the short time-interval between ablation and resection, or by the local electrical heterogeneity of tumour tissue, which requires further characterization of the optimal tissue- and tumour-specific ablation parameters to improve ablation success. These results represent an important step before IRE can be implemented as an ablation technique to treat central liver tumours. Alongside the other available tumour ablation techniques, IRE may become an important tool in the treatment of cancer in the near future. We plan to validate our results in the currently ongoing COLDFIRE-II trial (NCT01799044) for centrally located CRLM.
Chapter 4.1

References


4.2

Study protocol COLDFIRE-2: Colorectal liver metastatic disease: Efficacy of Irreversible Electroporation – a single-arm phase II clinical trial

Hester J Scheffer, Laurien GPH Vroomen, Karin Nielsen, Aukje AJM van Tilborg, Emile Fl Comans, Cornelis van Kuijk, Bram B van der Meijs, Janneke van den Bergh, Petrousjka (MP) van den Tol, Martijn R Meijerink
Abstract

Background
Irreversible electroporation (IRE) is a novel image-guided tumor ablation technique that has shown promise for the ablation of lesions in proximity to vital structures such as blood vessels and bile ducts. The primary aim of the COLDFIRE-2 trial is to investigate the efficacy of IRE for unresectable, centrally located colorectal liver metastases (CRLM). Secondary outcomes are safety, technical success, and the accuracy of contrast-enhanced (ce)CT and $^{18}$F-FDG PET-CT in the detection of local tumor progression (LTP).

Methods/design
In this single-arm, multicenter phase II clinical trial, twenty-nine patients with $^{18}$F-FDG PET-avid CRLM ≤ 3.5cm will be prospectively included to undergo IRE of the respective lesion. All lesions must be unresectable and unsuitable for thermal ablation due to vicinity of vital structures. Technical success is based on ceMRI one day post-IRE. All complications related to the IRE procedure are registered. Follow-up consists of $^{18}$F-FDG PET-CT and 4-phase liver CT at 3-monthly intervals during the first year of follow-up. Treatment efficacy is defined as the percentage of tumors successfully eradicated 12 months after the initial IRE procedure based on clinical follow-up using both imaging modalities, tumor marker and (if available) histopathology. To determine the accuracy of $^{18}$F-FDG PET-CT and ceCT, both imaging modalities will be individually scored by two reviewers that are blinded for the final oncologic outcome.

Discussion
To date, patients with a central CRLM unsuitable for resection or thermal ablation have no curative treatment option and are given palliative chemotherapy. For these patients, IRE may prove a life-saving treatment option. The results of the proposed trial may represent an important step towards the implementation of IRE for central liver tumors in the clinical setting.
Background

Colorectal cancer causes each 10th death due to cancer in Western countries. About 33% of all patients with colon cancer have liver-only metastatic disease. For these patients, surgical resection is the gold standard, with 5-year survival rates up to 60%. However, despite improvements of surgical techniques and current neoadjuvant chemotherapies, only 5-20% of patients with colorectal liver metastases (CRLM) can benefit from surgery due to number, localization, or distribution of tumors.

In light of the limitations of surgical resection for many hepatic tumors, a number of ablative technologies for liver-directed therapy have developed during the last 20 years. Of these techniques, thermal ablation using radiofrequency ablation (RFA) and microwave ablation (MWA) are most frequently used. A recent review showed a local recurrence rate of 2.8-29.7% of RF-ablated liver lesions at 12-49 months follow-up, and 2.7-12.5% of MW-ablated lesions at 5-19 months follow-up, with a 5-year survival rate around 30% for both techniques. Although thermal ablation has dramatically improved survival rates for patients with CRLM, factors like size and location limit its use and effectiveness. Efficacy of RFA rapidly decreases for lesions > 3cm. Also, the rate of complete tumor necrosis falls below 50% when vessels larger than 3mm abut the tumor as a consequence of the heat-sink effect. Ablation of lesions close to vital structures like major bile ducts and vessels is associated with a (substantial) risk of complications due to thermal damage.

Irreversible electroporation (IRE) is a novel ablation modality that may overcome some of the limitations of current thermal ablation therapies. It is based on a pulsating current created between multiple needle electrodes placed around the target lesion which alters the existing cellular transmembrane potential. If the duration of the applied electrical pulses is below the charging time of the outer cell membrane, an interaction of the electric field with subcellular structures occurs. This interaction results in permanent permeabilization of the cell membrane, which disrupts cellular homeostasis and ultimately leads to cell death. The irreversibly damaged cells are removed by the immune system. Two main factors stimulate research into IRE as an ablation modality. Since the mechanism of cell death is predominantly nonthermal, connective tissue structure is preserved, so there is no damage to associated blood vessels and bile ducts. For the same reason, treatment efficacy is not impeded by heat-sink. This allows for treatment of liver tumors deemed unresectable or ineligible for other local ablation techniques due to localization near these structures.

The capability of IRE to destroy CRLM in humans was recently demonstrated in the COLDFIRE-1 ablate-and-resect trial. In this trial resectable CRLM were treated with IRE, followed by resection one hour later. The investigators demonstrated cell death of the ablated tumors within one hour after IRE, with intact larger vascular and ductal structures within the ablation zone. The first studies investigating hepatic IRE on clinical indication also yield promising results. A systematic review found an overall complication rate of 16% for hepatic IRE, similar to RFA. However, the tumors treated with IRE were all located near thermally sensitive structures, as opposed to the thermally ablated lesions. Complete tumor eradication was achieved in 67-100%, and this percentage was even higher for tumors < 3cm. However, since most studies are retrospective with short-term follow-up using heterogeneous study populations and design, the value of the current evidence remains limited. Also, the optimal imaging modality for follow-up after IRE needs to be analyzed.
Chapter 4.2

The primary aim of the COLDFIRE-2 trial is to investigate the efficacy of IRE for CRLM that are unsuitable for resection and thermal ablation due to the vicinity of vulnerable structures such as bile ducts, vessels and bowel. Other outcomes are safety of IRE, and the accuracy of contrast-enhanced computed tomography (ceCT) and 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) CT in the detection of local tumor progression (LTP).

Methods/Design

Design
The COLDFIRE-2 trial is a phase II, multicenter, prospective single-arm trial that is organized by the VU University Medical Center in Amsterdam, the Netherlands. Patients will be recruited at three academic hospitals in the Netherlands (VU University Medical Center, Amsterdam; Academic Medical Center, Amsterdam; Leiden University Medical Center, Leiden). The study protocol has been approved by the Medical Ethical Review Board of the VU University Medical Center. The trial is investigator-sponsored, independent of industry and is registered at clinicaltrials.gov under number NCT02082782. The trial will be conducted in accordance with the Declaration of Helsinki. The inclusion and exclusion criteria for the COLDFIRE-2 trial are summarized in table 1.

Table 1: In- and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic proof of primary colorectal tumor</td>
<td>Ventricular cardiac arrhythmias</td>
</tr>
<tr>
<td>Radiologic proof of colorectal liver metastasis, unsuitable for resection and thermal ablation due to vicinity to vascular or ductal structures</td>
<td>Congestive heart failure, NYHA Class ≥ 3</td>
</tr>
<tr>
<td>18F FDG-PET avidity of target lesion and visible on ceCT</td>
<td>Active coronary artery disease</td>
</tr>
<tr>
<td>Lesion size ≤ 3.5 cm</td>
<td>History of epilepsy</td>
</tr>
<tr>
<td>Adequate bone marrow, liver and renal function</td>
<td>Any implanted stimulation device</td>
</tr>
<tr>
<td>ASA-classification 0-3</td>
<td>Chemotherapy &lt;6 weeks prior to treatment</td>
</tr>
</tbody>
</table>

Eligibility criteria
All patients are treated with curative intent and must have received previous chemotherapy for CRLM at some stage in their disease. Prior to inclusion, all patients will be discussed in a multidisciplinary liver tumor board consisting of a hepatogastroenterologist, hepatobiliary surgeon, medical oncologist, radiation oncologist, abdominal and interventional radiologist. Decision on treatment will be at their discretion. Patients who present with more than one metastasis can only be included if treatment with curative intent is still realistic and if all lesions can be treated during the same session or within six weeks after the IRE procedure. Thermal ablation or resection of concomitant lesions during the same session is therefore allowed and limited extrahepatic disease, defined as ≤5 nodules in the lung and/or one other metastatic site which is amenable to future definitive treatment, is not contra-indicated. All participants from all participating centers will provide written informed consent.

IRE procedure
Depending on concurrent treatment of other lesions (surgical resection or thermal ablation), patients will either be treated during an open procedure using intra-operative ultrasound (IOUS), or percutaneously using CT. Patients undergoing laparotomy will receive a thoracic
IRE for CRLM - the COLDFIRE-2 study protocol

epidural before surgery. For percutaneous procedures, to allow repeated and real-time visualization of both the tumor and the adjacent vessels, a catheter is placed within the common hepatic artery approaching from the right femoral artery. Technical details of this procedure have been previously described by Van Tilborg et al.\textsuperscript{19} All procedures will be performed under general anesthesia, induced with propofol, sufentanil, and rocuronium and maintained with propofol and remifentanil. First, the exact three-dimensional measurements of the target lesion are defined using IOUS or aortic catheter based CT. The planned electrode configuration must result in an expected geometry of the ablation zone that fully covers the tumor and a tumor-free margin of at least 5mm in all directions.\textsuperscript{20} Depending on the size, 2-6 needle electrodes (NanoKnife, AngioDynamics, Latham, NY) with an active working length of 20 mm are positioned in the outer border or just adjacent to the tumor under IOUS or CT guidance, aiming at an inter-electrode distance of 20 mm (±2 mm). All needles are placed parallel to each other to promote homogeneous energy delivery. After confirmation of correct distances with IOUS or with unenhanced CT using multiplanar reformating, ten test-pulses of 1500 V/cm and 90 μs are delivered via each electrode pair, after which the delivered current is verified. The target current lies between 20–50A and voltage settings are manually adjusted in case of over- or undercurrent. Subsequently, three cycles of 30 pulses are administered to reach a total of 100 pulses per electrode pair. If more than 6 electrodes are needed for larger tumors, electrodes are repositioned to perform overlapping ablations. Similarly, for tumors with a depth larger than 20 mm, after ablation of the deepest part of the tumor a 1.5 cm pullback of the electrodes is performed to treat the superficial part. Immediately after IRE, a control aortic catheter CT or IOUS is made to evaluate technical success and to exclude early complications. The next day, ceMRI is performed to verify patency of vascular and ductal structures, as well as technical success. In case of incomplete ablation on ceMRI, the suspected residual tumor will be retreated in the following weeks. A flow diagram of the trial is shown in figure 1.

**Imaging**

Four-phase liver CT and \( ^{18} \)F-FDG PET-CT will be performed before IRE, and 3, 6, 9 and 12 months after IRE. Imaging is conducted according to EANM guidelines with the same \( ^{18} \)F-FDG PET-CT in each participating center (Philips Gemini TF PET-CT system, Philips Medical Systems, Cleveland, Ohio, USA) [21]. Whole body \( ^{18} \)F-FDG PET (skull base to mid-thigh) starts 60 minutes after FDG injection, followed by a low-dose CT for attenuation correction and anatomical co-localization of \( ^{18} \)F-FDG PET-findings. Next, a diagnostic 4-phase CT of the liver is performed. Scanning
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parameters are shown in table 2.

| Table 2: Scanning parameters of 18F-FDG PET-CT and 4-phase liver CT |
|-------------------------|----------|------------|---------------|----------------|----------------|
|                        | Delay (sec) | Contrast | Matrix (mm2) | Slice thickness (mm) | Current (mA/slice) | Energy (keV) |
| Whole body PET         | 144x144   | 4x4       | 5            | 30-50              | 100              | 120          |
| Low-dose CT            | -         | 512x512   | 1x1.17       | 220 AEC            | 80              |
| 4-phase liver CT       | 100ml     | 512x512   | 0.68x0.68    | 300 AEC            | 120              |
| - Precontrast          |           | Xenetix300|              |                    | 175 AEC          | 120          |
| - Arterial             | 12        |           |              |                    | 450 AEC          | 80           |
| - Venous               | 30        |           |              |                    | 220 AEC          | 120          |
| - Hepatic              | 22u       |           |              |                    | 175 AEC          | 120          |

AEC, Automated exposure control

Treatment response is primarily based on a per-lesion analysis. The standard of reference is defined by a combination of 4-phase liver CT and 18F-FDG PET-CT, and, if available, histologically proven malignancy. Pathologically elevated carcinoembryonic antigen (CEA) is used when appropriate (e.g. if no other metastases are present).

Treatment response on 4-phase liver CT is assessed using the Response Evaluation Criteria In Solid Tumors (RECIST).22 The Positron Emission tomography Response Criteria In Solid Tumors (PERCIST) criteria are used for 18F-FDG PET-CT.23 On 4-phase liver CT, LTP is defined as a growing (> 20%; longest diameter; axial plane) hypodense lesion within 1cm of the ablation zone. On 18F-FDG PET-CT, locally increased FDG uptake within 1cm of the ablation region is considered an LTP.24 If LTP is suspected on both modalities during follow-up, the patient will be evaluated for re-treatment in our multidisciplinary liver tumor board. To establish the value of PET-CT and ceCT for the prediction of LTP the suspected area will be biopsied in the same session prior to re-treatment, although the suspicious lesion will be re-ablated regardless. In case of discrepancy between the 4-phase liver CT and 18F-FDG PET-CT findings a liver MRI will be performed as problem solver. If MRI is also inconclusive, multidisciplinary deliberation will decide upon either repeat imaging 3 months later, or biopsy of the suspected recurrence. If chemotherapy is indicated during follow-up (e.g. due to new metastatic disease), the patient will remain in follow-up. The trial will end twelve months after the last IRE procedure. If no LTP or new metastatic disease has been diagnosed at this moment, regular follow-up will be resumed consisting of 6-monthly 18F-FDG PET-CT and 4-phase liver CT.

Data collection and handling

The study coordinators (HS and LV) will collect the data. All data will be handled confidentially and anonymously. A subject identification code is used to link the data to the subject. The study coordinators safeguard the key to the identification code. The handling of personal data will comply with the Dutch Personal Data Protection Act.

To investigate the accuracy of 18F-FDG PET-CT and 4-phase liver CT in the diagnosis of LTP after IRE, twelve months after the last IRE treatment blinded data sets from all centers will be reviewed separately and independently by two designated radiologists (BvdM and
JvdB) and two nuclear medicine physicians (OH and EC) in a consensus reading. Each lesion will be scored on a separate form and results will be scored on a one to five scale (table 3). The reviewers will be blinded to the results of the other imaging modality and to the final oncologic outcome as determined by the standard of reference.

Table 3: Study form for reviewers’ results (per lesion)

<table>
<thead>
<tr>
<th>Study number</th>
<th>Definition</th>
<th>Reviewer number</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td></td>
<td>Confident that no tumor recurrence is present in the ablation zone</td>
</tr>
<tr>
<td>2</td>
<td>Probably benign</td>
<td></td>
<td>The appearance of the ablated lesion is compatible with post-ablational inflammation or rim-like characteristics</td>
</tr>
<tr>
<td>3</td>
<td>Equivocal</td>
<td></td>
<td>There is doubt whether the imaging features indicate local tumor progression or inflammation</td>
</tr>
<tr>
<td>4</td>
<td>Probably malignant</td>
<td></td>
<td>Confident about local progression in the ablation zone</td>
</tr>
<tr>
<td>5</td>
<td>Impaired quality</td>
<td></td>
<td>Quality of the images precludes adequate diagnosis</td>
</tr>
</tbody>
</table>

Comments:

Primary and secondary objectives

The primary endpoint of the COLDFIRE-2 trial is efficacy of IRE for CRLM. Secondary endpoints are progression-free survival (PFS) and overall survival (OS). Other secondary endpoints are safety, technical success, and the accuracy of 18F-FDG PET-CT and 4-phase liver CT in the detection of LTP after IRE.

Primary efficacy rate is defined as the percentage of target tumors successfully eradicated 12 months after the initial IRE procedure, according to the RECIST criteria and PERCIST criteria. Secondary efficacy rate is defined as the percentage of tumors successfully eradicated 12 months after the initial IRE procedure, including tumors that have undergone successful repeat ablation following identification of LTP.\(^ {26}\)

Progression-free survival is defined as the time from the first IRE procedure to the time of radiologic disease progression or death of disease. Overall survival is defined as the time from the IRE procedure until death of disease.\(^ {27}\) Both PFS and OS will be determined using the Metabolic Imaging And Marker Integration (MIAMI) criteria as proposed by Hosein and colleagues, which integrates anatomic response parameters (4-phase liver CT) with two functional parameters: PET activity and CEA levels.\(^ {26}\) For safety analysis, all major adverse events and all adverse events occurring during or within 12 months after IRE treatment will be recorded according to the Common Terminology Criteria of Adverse Events (CTCAE) v4.0.\(^ {29}\) Pain assessment using the visual analogue scale (VAS) and patient analgesic consumption will be recorded. Patency of vessels and bile ducts on follow-up cross-sectional imaging will be analyzed to identify late complications. Technical success is defined as (1) the ability to successfully deliver all planned pulses according to protocol and (2) complete lesion coverage on post-procedure ceCT or IOUS, and 24-48h post-procedure MRI.\(^ {26}\)

Sample size calculation and statistical considerations

Based on the current literature our hypothesis is that 10% of the treated tumors will recur after the initial treatment (primary technique efficacy 90%), which implies a local recurrence rate of 0.1.\(^ {5,30}\) Choosing a target width of 0.25 with \(p \leq 0.05\), we used the confidence interval
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formula "Exact" for two-sided confidence intervals for one proportion (Clopper-Pearson).\textsuperscript{35, 32} With this formula, actual width is 0.249, with a 95% confidence interval of 0.020 – 0.269. This calculation results in a sample size of 29 patients. Considering a 5% loss to follow-up, the total number of patients needed is 31.

All clinicopathological and procedural variables will be described and analyzed. Continuous variables will be summarized with standard descriptive statistics including means, standard deviations, medians and ranges. Categorical variables will be summarized with frequencies. P-values ≤ 0.05 will be considered statistically significant. Univariate survival analysis will be performed using the Kaplan-Meier method. Differences in local recurrence rate between subgroups like tumor size and adjuvant chemotherapy will be analyzed descriptively since the expected number of recurrences is too small for statistical analysis.

The accuracy of "F-FDG PET-CT and 4-phase liver CT in diagnosing local recurrences will be investigated by comparison to the reference standard. Interobserver-variability will be determined using Cohen’s Kappa. Sensitivity and specificity of both techniques will be calculated with their respective 95% confidence intervals. McNemar’s test is used to assess a statistically significant difference between both imaging modalities.

Discussion

IRE for colorectal liver metastases

New cancer treatments are typically best defined from phase III randomized trials comparing the investigated therapy with the current standard. However, in the field of local tumor ablation, this has proven a difficult challenge. Since its introduction decades ago, the number of randomized trials remains limited. An attempt to organize a trial comparing RFA to surgical resection has failed (French FFCND 2002-02) and it is unlikely that another trial can be organized in the near future.\textsuperscript{33} The current literature on the clinical application of IRE is scarce with no randomized controlled trials. However, because the indication for IRE lies with tumors in which no other suitable local therapy is available, a randomized trial comparing IRE to e.g. surgical resection or thermal ablation is not unrealistic at this point in time.

Results of single-arm studies on hepatic IRE report a high variation in efficacy, with local recurrence rates between 67-100%.\textsuperscript{17} Similar to RFA, efficacy is higher for tumors ≤ 3cm. However, since IRE is only used as a ‘last resort’ curative treatment option in patients that would otherwise receive palliative chemotherapy, these early efficacy rates are promising and encourage larger prospective studies.

Feasibility

Intraprocedural monitoring and control of ablation play a critical role in the success of tumor ablation.\textsuperscript{34} The feasibility of real-time monitoring with US during hepatic IRE has been demonstrated in both animal and human studies. The ablation zone immediately appears as a hypoechoic area with well-defined margins.\textsuperscript{16,35,36} Immediate postprocedure ceCT also shows a well-defined hypodense area on the portal venous phase with variable periablational hyperenhancement.\textsuperscript{37,38} Size and shape of the IRE ablation zone on both US and CT has proven to correlate reliably with the pathologically defined zone of cell death.\textsuperscript{36,37} These imaging modalities could therefore be used to ensure that the realm of ablation encompasses the originally targeted volume with a sufficient margin,\textsuperscript{15} which is a focus of the presented trial.
Imaging follow-up

The main concern following tumor ablation is the risk of developing LTP. Early diagnosis of LTP is imperative because repeated treatment can still effectuate complete tumor clearance, especially for smaller recurrences. CeCT is the current mainstay of staging and follow-up.39 One shortcoming of ceCT in the monitoring of post-ablative lesions for recurrent disease is the presence of post-ablation effects. Because reactive tissue and viable tumor can both present as hypodensity around the ablated lesion, their distinction can be difficult.24 Due to the visualization of increased glucose metabolism in tumor cells, 18F-FDG PET has proven a very sensitive and accurate tool for the diagnosis of tumor manifestations in patients with colorectal carcinoma.39 Using 18F-FDG PET-CT, 18F-FDG PET images are combined with CT in a single session. Anatomical and morphological information from CT is used to increase the precision of localization, extent, and characterization of lesions detected by 18F-FDG PET.21 Several studies have shown the superiority of 18F-FDG PET-CT over morphologic imaging alone in the follow-up after ablation of CRLM: sensitivity and specificity for the detection of LTP are 92% 100% for 18F-FDG PET-CT compared to 83% and 100% for ceCT.24 However, the diagnostic accuracy of 18F-FDG PET and 18F-FDG PET-CT is strongly affected by chemotherapy, so for patients receiving chemotherapy during follow-up after IRE ceCT may prove the most reliable imaging modality.

After IRE, immediate ceCT shows a hypodense ablation zone that does not enhance post-contrast. A transient peripheral rim of contrast enhancement can be present, representing hyperemia.19 In the months after ablation, the ablation zone slowly decreases in size and should not show uptake of contrast. PET scans show a dynamic response to the IRE ablation. Three days following IRE, an 18F-FDG-avid peripheral zone surrounding the ablated region appears. This initial increase in uptake at the periphery of the IRE region may be explained by an inflammatory response, increasing metabolic activity at the targeted region as the cellular debris is removed from the ablation site.19 In our experience the inflammatory response can persist for several months, which renders evaluation of the ablation zone with 18F-FDG PET difficult. On the contrary, ablated lesions that show focal rather than rim-like uptake in the periphery are considered suspect for local recurrence. Because much is still unknown about the imaging characteristics of liver lesions treated with IRE, standardized follow-up regimens are lacking. The COLDFIRE-2 trial focuses on the typical imaging characteristics of electroporated CRLM over time. The gold standard for LTP is histologic confirmation. With biopsies taken from all suspicious lesions prior to re-treatment, the trial also assesses the accuracy of the pre-defined definition of LTP on PET-CT and ceCT.

A potentially curative treatment option for a group of patients that is currently offered chemotherapy with palliative intent is of major importance. IRE may prove a safe and valuable fortification in the armory of interventional oncologists treating patients with liver tumors. The aim of the COLDFIRE-2 trial is to contribute to the available evidence on IRE with respect to safety, efficacy, imaging characteristics and follow-up guidelines. The results of the proposed trial may represent an important step towards the implementation of IRE for central liver tumors in the clinical setting.
Chapter 4.2

References


4.3

Irreversible electroporation for colorectal liver metastases: tricks of the trade

Hester J Scheffer, Marleen CAM Melenhorst, Ana Echenique, Karin Nielsen, Aukje AJM van Tilborg, Willemien van den Bos, Laurien GPH Vroomen, Petrousjka (MP) van den Tol, Martijn R Meijerink

Techniques in Vascular and Interventional Radiology 2015;18(3):159-69
Abstract

Image-guided tumor ablation techniques have significantly broadened the treatment possibilities for primary and secondary hepatic malignancies. A new ablation technique, irreversible electroporation (IRE), was recently added to the treatment armamentarium. As opposed to thermal ablation, cell death with IRE is primarily induced using electrical energy: electrical pulses disrupt the cellular membrane integrity, resulting in cell death whilst sparing the extracellular matrix of sensitive structures such as bile ducts, blood vessels and bowel wall. The preservation of these structures makes IRE attractive for colorectal liver metastases (CRLM) that are unsuitable for resection and thermal ablation due to their anatomic location. This review discusses different technical and practical issues of IRE for CRLM: the indications, patient preparations, procedural steps and different “tricks of the trade” used to improve safety and efficacy of IRE. Imaging characteristics and early efficacy results are presented. Much is still unknown about the exact mechanism of cell death and about factors playing a crucial role in the extent of cell death. At this time, IRE for CRLM should only be reserved for small tumors that are truly unsuitable for resection or thermal ablation due to abutment of the portal triad or venous pedicles.
IRE for CRLM - tricks of the trade

Introduction
The past decades advances in technology have fuelled interest in less invasive treatment options for solid tumors. The rapid development of different image-guided ablation techniques has considerably broadened the therapeutic possibilities for surgically incurable colorectal liver metastases (CRLM). For metastases that are neither amenable for resection, nor for thermal ablation with radiofrequency or microwave ablation due to their vicinity to blood vessels or bile ducts, irreversible electroporation (IRE) is increasingly used. The working mechanism of IRE is based on electrical energy; high voltage electrical pulses cause irreversible cellular membrane disruption, leading to cell death. At the same time, the underlying connective tissue scaffold should remain intact. Although the development of heat is an inevitable side effect of the electrical pulses, this temperature increase is not believed to be detrimental to the surrounding connective tissue. As a result, inlaying vulnerable structures such as bile ducts and blood vessels remain patent. A second advantage of IRE is that, unlike concurrent ablation techniques, its efficacy is not impeded by convective cooling of neighboring blood vessels (the 'heat-sink effect'). The current strength of hepatic IRE lies with small tumors in the proximity of major vascular structures or portal pedicles where heat-sink and collateral damage must be avoided for maximum safety and efficacy. The application of IRE for this indication as a last resort for curative treatment is worldwide gaining popularity. This review serves as a practical approach for the treatment of CRLM with IRE and should aid interventional radiologists performing IRE for this indication.

Clinical evaluation and indications
Irreversible electroporation for CRLM is currently only indicated for those tumors that are unsuitable for surgical resection and thermal ablation. Most frequently this applies to centrally located liver tumors. Similar to surgical resection, the general criterion for image-guided ablation is that it is performed with curative intent, which means that all tumors must be suitable for some kind of local treatment. Concurrent treatment of additional tumors in the same treatment session by e.g. resection or thermal ablation is therefore not uncommon. IRE is repeatable and can be used to treat residual disease as well as new lesions.

Although there are no strict size criteria, similar to RFA, IRE appears to be most effective for tumors <3 cm in diameter; beyond this size treatment efficacy quickly decreases and may require staged therapy with multiple ablation sessions. Similarly, there is no absolute number of tumor eligibility, but most series agree that patients with more than four simultaneous liver metastases are suboptimal candidates for image-guided percutaneous ablation. Besides size and number of the lesions, factors like age, performance status, comorbidity and

Figure 1 Percutaneous CT-guided (left) and open (right) IRE-procedure
Chapter 4.3

previous oncologic treatment all play part in the assessment of a patient’s suitability for local treatment. Given the versatility in CRLM treatment, the indication for IRE should be discussed in a multidisciplinary liver tumor board, consisting of at least a radiologist, interventional radiologist, surgical oncologist, medical oncologist, hepato-gastroenterologist, and radiation therapist.

For IRE, specific contra-indications apply, such as an inability to undergo general anesthesia. Careful cardiac screening and full anesthetic review is mandatory.⁶ The high-voltage electrical pulses could theoretically induce arrhythmias in a patient with a history of (ventricular) arrhythmias or a pacemaker, which are other contra-indications.

Preprocedural imaging

Thorough review of cross-sectional imaging is critical to assess the exact size and shape of the lesion and its vicinity to other structures such as bile ducts and blood vessels. Ideally, contrast enhanced (ce) computed tomography (CT) is performed within 30 days prior to ablation, combined with low-grade 18fluorine deoxyglucose positron emission tomography (¹⁸F FDG-PET-) CT. The ceCT provides valuable information regarding tumor location, size and geometry that are essential for treatment planning. Baseline FDG-PET is used to confirm the metastatic nature of liver lesions and to exclude extrahepatic disease. During follow-up PET has great value in determining tumor viability after ablation and early detection of local treatment site recurrences.⁷

Equipment needed

The NanoKnife (AngioDynamics, Latham, NY), a low-energy direct-current electroporation device, is currently the only commercially available IRE system. It consists of a generator, footswitch, and 19 gauge unipolar needle electrodes with an active working length that can be varied from 5mm to 40mm.⁸ To prevent pulse-induced arrhythmias, the Accusync electrocardiogram (ECG)-gating device (model 72; Milford, Connecticut) is connected to a five-leads ECG, which synchronizes pulse delivery within the refractory period of the heart (the R-wave on the ECG). Just before the start of IRE delivery, complete muscle relaxation must be ensured to prevent generalized muscle contractions. Electroporation can be performed during laparotomy using intra-operative ultrasound (IOUS) or percutaneous using CT-fluoroscopy and US-guidance (figure 1). Because CT enables multiplanar reconstruction of the tumor in relation to the surrounding structures and the needle electrodes, we prefer the use of CT for percutaneous procedures.
Procedural steps

Electrode configuration and placement

First, with the patient under general anesthesia and in the supine or left lateral position, the exact geometric measurements of the target lesion are assessed using either IOUS or cCT, which determines the number and configuration of the electrodes (figure 2). The maximum number of electrodes that can be used simultaneously is six. For hepatic IRE the active working length of the electrodes is set at 20mm. Based on in vivo animal studies on the porcine liver, an inter-electrode distance of 20mm is considered the most effective treatment distance at which the created ablation zone has an oval shape.9

Ideally, electrodes are placed almost precisely parallel to each other (maximum angulation 10°) to promote homogeneous energy delivery. The planned configuration should result in an expected geometry of the ablation zone that fully covers the tumor and a tumor-free margin of at least 5mm in all directions.10 Because the calculated ablation zone extends at least 5mm outwards from the electrodes, they should be placed in the outer border or just adjacent to the tumor (figure 3).11

IRE ablation

When all needles are in place, correct interelectrode distances are confirmed with non-enhanced CT using multiplanar reformatting or with IOUS.12 Electroporation is subsequently performed between all electrode pairs that are separated within the minimum (1.5cm) and maximum (2.4cm) distance from each other, including diagonals. For example, for a four-
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needle configuration, the maximum number of combinations used is six, but this depends on the shape of the tumor. First, ten test-pulses of 1500 V/cm with a duration of 90μs are delivered for each vector, after which the delivered current is verified. The target current lies between 20-50Α and voltage settings are manually adjusted in case of over- or undercurrent. Subsequently, for each electrode pair 80 remaining pulses are administered to reach a total of 90 pulses per vector. After IRE, the resulting mean voltage and current of each vector are shown in the display. If more than 6 electrodes are needed for larger tumors, electrodes can be repositioned to perform overlapping ablations. Similarly, for tumors with a depth more than 20mm, after ablation of the deepest part of the tumor a 1.5cm pullback of the electrodes is performed to ablate the superficial part of the tumor.

Ablation monitoring

Intraprocedural monitoring and control of ablation play a critical role in the success of tumor ablation. The feasibility of real-time monitoring during hepatic IRE has been demonstrated in both animal and human studies. On ultrasound, a 1- to 2-mm hyperechoic area with gas typically forms around the tip of the electrodes (figure 4), which is thought to be caused by the electrolysis of water (H$_2$O) into oxygen (O$_2$) and hydrogen gas (H$_2$) by the electric current passing through the tissue. However, heat-induced evaporation around the electrodes has also been described. Studies investigating IRE on healthy porcine liver proved that the size and shape of the ablation zone on both US and CT correlates reliably with the pathologically defined zone of cell death. Therefore, these imaging modalities could be used to ensure that the realm of ablation encompasses the originally targeted volume with a good margin.

Tricks of the trade

![Figure 4](image-url)

Figure 4: Intra-operative ultrasound during open IRE of a CRLM. (A) Electrodes placed on either side of the tumor. (B) Hyperechoic area surrounding the electrodes caused by gas formation. (C) Hypoechoic area around the tumor after the ablation.

Precise electrode placement lies at the basis of a successful ablation. Misplacement by a margin of millimetres can result in residual tumor, so accurate planning as well as reliable intraprocedural visualization of the target lesion is crucial. Besides taking into account rib and bowel position within the puncture plane, traversing vital structures must be avoided. Owing to the relatively small size of the ablation zone created between two electrodes (approximately 1.5cm in shortest axis), multiple probes are commonly used. The task of precise alignment of multiple electrodes presents a new level of challenge even for the experienced interventional radiologist.
Electrode steering
Because the 19 gauge electrodes are remarkably floppy and CRLM are more solid than normal liver tissue, it can be exceedingly difficult to correct a deviating approach (figure 5A). Simply correcting the angle will cause the probe to bow, resulting in a tip deviation even further away from the target (figure 5B). One solution is to angulate the electrode in the opposite direction so that upon advancing, the electrode will follow a curved path towards the target (figure 5C).

Optimizing target visibility
Tumor tissue and ablation zones are often barely visible on unenhanced CT especially when they have been pre-treated with chemotherapy. During CT-guided IRE, the delineation of tumor, surrounding vessels and the induced coagulation zone is often limited to a time window after application of intravenous contrast material. Consequently, if the maximum dose of contrast is reached after one or two injections required for treatment planning prior to the procedure, repetitive intraprocedural monitoring is restricted. This is a major drawback because dynamic and real-time tumor and vessel delineation is key to safe and precise probe placement. One method to reduce the contrast dose is bolus chasing. This allows pre- and postablation contrast imaging for all ablative modalities. To further improve intraprocedural lesion and vessel conspicuity, we recently demonstrated the feasibility of transcatheter CT hepatic angiography (CTHA) with percutaneous liver tumor ablation. The injection of contrast directly into the proper hepatic artery enables repeated contrast-enhanced imaging and real-time CT fluoroscopy, which improves lesion conspicuity and also provides real-time information on the vicinity of blood vessels. Immediately after IRE, the ablated area is clearly delineated, with the typical appearance of the avascular ablation zone surrounded by a hypervascular rim (figure 6).

Complications
Puncture related complications such as pneumothorax and hemorrhage are unfrequently encountered and are comparable to other needle-guided liver interventions. The electric fields applied in IRE can cause cardiac arrhythmias, but synchronized pulsing with the heart rhythm greatly reduces this risk. A recent systematic review of the literature on IRE showed that with cardiac gating only minor arrhythmias occurred (incidence 2.2%). The overall complication rate for hepatic IRE was 16%, but these were all minor complications. Pain appears similar to pain after thermal ablation. With a 2.5% risk of hepatobiliary complications such as portal vein thrombosis and bile duct leakage or occlusion, the preservation of these structures seems probable, especially considering that IRE was mostly performed on tumors near or around portal pedicles.

In the past years, several studies have demonstrated that with IRE, the generation of at least
some heat has proven an inevitable but definite side effect. This increase in temperature
is highest immediately around the electrodes. Moreover, by using an infrared camera
to visualize the thermal electrode-tissue interactions in gel, our research team recently
discovered that the negative electrode gets warmer than the positive electrode (figure 7;
unpublished data). To prevent unintended damage when ablating near thermally sensitive
critical structures we therefore recommend avoiding placement of the electrodes less than
2mm to central bile ducts or large blood vessels. If placement of an electrode near vulnerable
structures is however inevitable, we recommend that this should be the positive electrode to
minimize the chance for thermal damage.

Given the fact that the underlying rationale for the current and future clinical application
paradigms of IRE are in large part based on the assumption of the non-thermal nature of IRE,
further characterization of potential thermal effects of IRE is warranted to ensure safe clinical
application and optimal treatment planning.

The vicinity of metallic objects such as stents in the ablation zone might change the electric
field distribution, resulting in an unpredictable ablation zone and heating of the metal.
For this reason placement of electrodes near or surrounding metallic objects like stents is
discouraged by the manufacturer. However, several centers including ours safely performed

Figure 6: (A) Transcatheter ceCT (CTHA)
showing a small non-attenuating CRLM (arrow)
adjacent to the middle hepatic vein. (B) PET-CT
pre-IRE showing the FDG-avid lesion (arrow).
(C) CT fluoroscopy with two electrodes in situ.
(D) CTHA immediately post-IRE showing a large
non-enhancing ablation zone surrounding the
lesion (arrow) with peripheral hyperattenuating rim. (E) ceCT 2 weeks post-IRE showing
shrinkage of the hypodense ablation zone.
(F) Coronal MPR of ceCT 2 weeks post-IRE. (G)
ceCT 3 months post IRE demonstrating further
shrinkage of the non-enhancing ablation zone.
(H) PET-CT 3 months post-IRE showing absence
of tracer uptake of the treated lesion.
IRE with a metal stent within the ablation zone. This sometimes resulted in high current requiring voltage adjustments during treatment. The influence of metallic objects on the electric field distribution and subsequent ablation zone should be further explored.

**Future developments**

To ensure the long-term success of IRE, further efforts are needed to reduce the chance of live tumor cells remaining within the ablation zone. During electroporation, cell membrane permeability leads to an increase in tissue conductivity and depends on strength, number, and duration of the pulses. Animal studies have shown that the conductivity changes are one of the factors that determine ablation success. They could therefore provide real-time feedback on ablation progression and treatment outcome.\(^{25, 26}\)

For multiple electrode arrays, Appelbaum et al recently discovered that rather than applying all 90 pulses sequentially to each of the six electrode pairs in a four-probe array, multiple shorter cycles of pulse application enable a greater effect with larger ablation zones.\(^{27}\) Indeed, the authors demonstrated that cyclical pulse application leads to higher electrical conductivity, possibly depicting increasing ‘leakiness’ of the cellular membranes. Moreover, cyclic pulsing may also result in a lower temperature rise, decreasing the risk of thermal damage.

Abovementioned findings are all based on animal studies investigating the effect of IRE on healthy liver tissue. Electric field dose-response studies for tumor-specific tissues are scarce, and more research on the electric properties of malignant tissues with irregular geometries is needed to identify the optimal electric field and ablation settings for maximized ablation of CRLM, as well as other tumor types.

**Clinical follow-up**

CT and MRI are the most commonly used imaging methods to monitor postablative lesions for remnant or recurrent disease after hepatic RFA and MWA.\(^7\) Several studies have shown the superiority of PET-CT over morphologic imaging alone in the follow-up after thermal ablation of CRLM with a sensitivity and specificity of PET-CT (92% and 100%) compared to ceCT (83% and 100%) regarding the detection of local tumor progression.\(^7\)
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Much is still unknown about the imaging characteristics of liver lesions treated with IRE. As a consequence, standardized follow-up regimens are lacking. To investigate the typical appearance of electroporated CRLM, we have performed regular ceCT, PET-CT and magnetic resonance imaging (MRI) during follow-up.

Figure 8: MR images of a central CRLM treated with IRE. (A-C) CE T1-weighted, T2-weighted, and DWI image of a lesion prior to IRE. (D-E) MRI 1 day post-IRE demonstrating a hypointense ablation zone with hyperintense rim on T2 and an enhancing rim on CE T1. (F) DWI 1 day post-IRE with diffusion restriction of the ablated area especially at the periphery and reduced diffusion restriction of the ablated lesion (arrow). (G-L) CE T1, T2 and DWI images 2 weeks and 3 months post-IRE demonstrating resolution of the ablated area.
CT
Post-IRE ceCT is used to ensure that the realm of ablation encompasses the originally targeted volume with a good margin and to exclude complications. Immediately after IRE, the ablation zone appears hypodense and can show an enhancing peripheral rim. Follow-up CT imaging at 4 to 6 weeks is performed to exclude new sites of disease and local disease progression. Realistically, it is difficult to exclude local progression this early after IRE on CT as CRLM typically do not enhance unless there are additional sites of involvement or significant increase in the size of the postablation hypodense lesions. In the months after ablation, the ablation zone slowly decreases in size and should not show uptake of contrast (figure 6G).

PET
PET scans show a dynamic response to the IRE ablation. At three days following IRE, an FDG-avid peripheral zone surrounding the ablated region appears. This initial increase in uptake at the periphery of the IRE region may be explained by an inflammatory response, increasing metabolic activity at the targeted region as the cellular debris is removed from the targeted site. For PET-avid lesions, we have found PET-CT obtained within 24 hrs after IRE useful to assess completeness of ablation, which at this point in time must show absence of tracer-uptake within the ablated region.

In our experience, the inflammatory response visible as increased rim-like tracer uptake at the periphery of the lesion can persist for several months, which renders evaluation of the ablation zone difficult. However, ablated lesions, which show focal uptake rather than rim-like uptake in the periphery, are considered suspect for local recurrence.

MRI
One day post-IRE, T1-weighted contrast-enhanced MRI demonstrates a non-enhancing hypointense center and a slightly enhancing peripheral rim (figure 8D). T2-weighted MRI of the ablated region typically shows a hypointense center, surrounded by a hyperintense reactive rim caused by edema (figure 8E). Diffusion-weighted imaging (DWI) b800 shows a similar appearance (figure 8F). The radiologic ablation zone measurements show a high correlation with the histologically confirmed ablation zone in a study on IRE in rodent liver (P < .001 for both T1- and T2-weighted measurements) and could therefore also be useful as an indicator for complete or incomplete ablation and for follow-up evaluation of clinical outcome. As for CT and PET, when evaluating immediate post-IRE outcome, care should be taken that the hyperemic rim is not confused with regions of residual tumor, which would demonstrate focal and irregular peripheral enhancement.

Response evaluation criteria
A major challenge in reporting results on IRE for CRLM is the lack of uniform response criteria that can fully capture the efficacy of the procedure. Specific periprocedural imaging guidelines are needed to reassure the interventional radiologist when complete tumor ablation has occurred. A new response assessment system specific for CRLM was recently proposed, which can also be applied to ablative and transarterial modalities: the MIAMI criteria (table 1). The value of these criteria lies in the combination of anatomic response parameters using the RECIST criteria and two functional parameters: PET-activity and CEA levels. The application of the MIAMI criteria stratifies patients into two groups: those who have clinical benefit (complete response, partial response and stable disease) and those who
have no clinical benefit (progressive disease). The efficacy of IRE for CRLM was investigated in this study, and when the MIAMI criteria were applied, patients who showed clinical benefit exhibited significantly longer survival than patients who did not show clinical benefit (P < .018). Clearly, these criteria need validation in larger studies before they can be recommended for clinical application.

**Table 1: Proposed MIAMI criteria**

<table>
<thead>
<tr>
<th>Detail</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>Stable Disease</th>
<th>Progressive Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of longest dimensions on CT (RECIST)</td>
<td>Any decrease or &lt; 20% increase in target lesion(s)</td>
<td>Any decrease or &lt; 20% increase</td>
<td>Any decrease or &lt; 20% increase</td>
<td>≥ 20% increase or any new lesions</td>
</tr>
<tr>
<td>SUVmax on PET/CT scan (PERCIST)</td>
<td>Resolution of FDG uptake in target lesion(s)</td>
<td>≥ 30% decrease</td>
<td>No new lesions with ± 30% change in SUVmax</td>
<td>New abnormal FDG-avid lesions</td>
</tr>
<tr>
<td>CEA level after therapy</td>
<td>Normalization of CEA level</td>
<td>≥ 50% decrease</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Criteria required*</td>
<td>RECIST plus either PERCIST or CEA</td>
<td>RECIST plus either PERCIST or CEA</td>
<td>One of two</td>
<td>One of two</td>
</tr>
</tbody>
</table>

MIAMI: Metabolic Imaging And Marker Integration, PERCIST: Positron Emission Tomography Response Criteria in Solid Tumors, RECIST; Response Evaluation Criteria In Solid Tumors, SUVmax: maximum standardized uptake value. * If CT is the only evaluation modality available, the MIAMI response will be the same as the RECIST response. If all three modalities are available and there is discordance, the RECIST and PERCIST response takes precedence over the CEA response.

**Clinical results**

IRE is currently only used as 'last resort' curative treatment in patients that would otherwise receive chemotherapy with palliative intent. Early efficacy ranges widely between 55-93% in the published studies (table 2). For tumors < 3cm, efficacy is significantly better and tumors near large vessels do not recur more frequently, which suggests that the cellular destruction mechanism is indeed not impeded by heat-sink. However, current local control rates appear inferior to thermal ablation and surgical resection, especially for larger lesions.

Larger studies are needed to confirm these observations. Technique efficacy and the oncological outcome are momentarily under investigation in the currently recruiting prospective COLDFIRE-II trial (registered under NCT02082782 on clinicaltrials.gov).

**Table 2: Overview of clinical studies investigating efficacy of IRE for hepatic tumors**

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients (n)</th>
<th>Lesions (n)</th>
<th>Size (cm, median, range)</th>
<th>Tumor location</th>
<th>Approach</th>
<th>Median follow-up (months)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hosein et al.</td>
<td>29</td>
<td>58</td>
<td>2.7 (1.2-7.0)</td>
<td>Proximity to vascular structures or bowel</td>
<td>Percutaneous</td>
<td>11</td>
<td>79</td>
</tr>
<tr>
<td>Kingham et al.</td>
<td>28</td>
<td>65</td>
<td>1.0 (0.5-5.0)</td>
<td>57% ≤ 1 cm major hepatic vein, 40% ≤ 1 cm major portal pedicle</td>
<td>Open (22)</td>
<td>Percutaneous (6)</td>
<td>6</td>
</tr>
<tr>
<td>Silk et al.</td>
<td>9</td>
<td>19</td>
<td>3.0 (1.0-4.7)</td>
<td>14% &lt;1cm CBD, 68% &lt;1cm primary bile duct</td>
<td>Percutaneous</td>
<td>9</td>
<td>55</td>
</tr>
</tbody>
</table>

CBD: common bile duct
Conclusion
IRE offers a safe and valuable fortification in the armory of interventional oncologists treating patients with CRLM. Although the technique shows promise in clinical practice, it is still in its infancy, and we are just starting to understand the exact working mechanism of IRE. Technical improvements of the ablation device and increasing knowledge about tissue-specific electrical properties may result in improved efficacy in the future.

At this time, IRE should be reserved for well-selected patients with relatively small CRLM that are truly unsuitable for resection and thermal ablation. In general this means tumors abutting the portal triad or the hepatic venous pedicle, where thermal ablation is considered unsafe and less effective.
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Literature


Irreversible electroporation of a large centrally located hepatocellular adenoma in a woman with a pregnancy wish: a case report

Hester J Scheffer, Marleen CAM Melenhorst, Aukje AJM van Tilborg, Karin Nielsen, Karin MJ van Nieuwkerk, Richard A de Vries, Petrousjka (MP) van den Tol, Martijn R Meijerink

**Abstract**

Irreversible electroporation (IRE) is a novel image-guided ablation technique that is rapidly gaining popularity in the treatment of malignant liver tumors located near large vessels or bile ducts. We describe a 28-year-old female patient with a 5cm large, centrally located hepatocellular adenoma who wished to get pregnant. Regarding the risk of growth and rupture of the adenoma caused by hormonal changes during pregnancy, treatment of the tumor was advised prior to pregnancy. However, due to its central location the tumor was considered unsuitable for resection and thermal ablation. Percutaneous CT-guided IRE was performed without complications and led to rapid and impressive tumor shrinkage. Subsequent pregnancy and delivery went uncomplicated. This case report suggests that the indication for IRE may extend to the treatment of benign liver tumors that cannot be treated safely otherwise.
**Introduction**

Hepatocellular adenoma (HCA) is a relatively uncommon benign liver tumor that occurs mainly in women during their reproductive years. During pregnancy, hormone-induced growth of a pre-existing HCA can lead to spontaneous hemorrhage or rupture that may threaten the life of both mother and child. Because of its unpredictable behavior and the high mortality associated with rupture, women with a large (> 5cm) or growing and hormone-sensitive adenoma are generally advised to either avoid pregnancy or to undergo invasive treatment such as surgical resection, radiofrequency ablation (RFA), or embolization of the tumor prior to pregnancy.\(^2\)\(^-\)\(^4\)

Irreversible electroporation (IRE) is a novel tumor ablation technique that relies on electrical energy to achieve cell death. Multiple short, high-voltage electrical pulses are delivered to tumor tissue, which disrupts the cellular membrane and ultimately leads to cell death through apoptosis. IRE selectively destroys all cells within the treatment zone, whilst the integrity of the extracellular supporting tissue is preserved.\(^5\)

We present a young woman with a large, centrally located hepatocellular adenoma that was successfully treated with IRE.

**Case presentation**

A 28-year-old day-care nanny with a desire to conceive a child presented with a 52x40x40 mm large, biopsy proven HCA, stable in size two years after discontinuation of oral contraception. Because the lesion was located directly around the portal triad (figure 1A, 1B), the multidisciplinary teams of two academic hospitals considered the lesion unsuitable for resection and thermal ablation. Although the adenoma was difficult to detect on digital subtraction angiography, a trans-arterial embolization was attempted via small branches from the right hepatic artery (originating from the superior mesenteric artery) and via branches from the left hepatic artery using polyvinyl alcohol particles (Contour PVA 150-250, Boston Scientific, Natick, Massachusetts, USA). However, after two embolization procedures tumor volume (66.8 cm\(^3\)) and enhancement pattern remained unchanged. Due to the risk of rapid growth and subsequent rupture of the tumor during pregnancy, the patient was advised to avoid pregnancy. Subsequently, she contacted our hospital to inquire about the possibility to perform IRE. In a multidisciplinary meeting the risk of IRE close to the portal triad was carefully balanced against the risk of invasive treatment or potentially life-threatening hemorrhage during pregnancy. After due consideration, she opted for IRE treatment.

First, to allow repeated and real-time visualization of both the tumor and the vessels within the portal triad, a pigtail catheter was placed in the supra-celiac abdominal aorta. Technical details of this procedure have been previously described.\(^6\) Next, the patient was put in the supine position under general anesthesia. An aortic catheter-based contrast-enhanced (CE) CT scan (Sensation 64 slice MDCT, Siemens, Erlangen, Germany) was made by injection of a 40cc bolus of 1:1 saline diluted contrast material at 4cc/sec with a scan delay of 7 and 18 seconds for the arterial and portal-venous phase respectively. The three-dimensional measurements of the tumor (length 40mm, width 40mm and depth 52mm) and its proximity to other structures determine the number and configuration of the electrodes. The preferred distance between electrode pairs for optimally effective treatment is 20mm (± 5mm).\(^7\) All
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Figure 1: Images of the tumor before, during and after IRE. (A) Axial and (B) coronal T2 weighted MRI pre-IRE showing the slightly hyperintense lesion central in the right liver lobe in close proximity to the bile ducts (arrow in b: common bile duct). (C) Axial CT image of two electrodes placed into the adenoma. (D) Axial CE CT immediately post-IRE showing a typical ablation zone with a non-enhancing center and peripheral enhancing rim. (E) Axial T2 weighted MRI two weeks post-IRE depicting central edema in the ablation zone. (F) Coronal CE MRI (T1 with fat suppression) with minimal enhancement demonstrating a small remnant of the adenoma, needle tracks (asterisk) and patent bile ducts (arrow: common bile duct). Axial T2 weighted image eight weeks (G) and nine months (H) post-IRE with further reduction in tumor size.

were delivered along each vector after a planned 20mm pullback of the electrodes.

After removal of the electrodes CECT showed a remarkable hypo-attenuating ablation zone with the lesion encapsulated by a hyper-attenuating rim, reaching the confluence of the bile ducts (figure 1D). The procedure was completed without complications. The patient was discharged the next day after which subsequent recovery was uneventful. Follow-up MRI after 2 weeks showed lesion volume shrinkage with central edema on all sequences to 14.9 cm3 (22% of original 66.8 cm3, figure 1E). Hepatic artery, portal vein and central bile ducts were all patent (figure 1F). Eight weeks post-IRE the edematous ablation zone had further decreased to 10% of the original tumor volume on T2 (6.9 cm3, figure 1G). On T1, there was pulses were delivered in the absolute refractory period of the heart with use of electrocardiographic synchronization (Accusync, Model 72, Milford, Connecticut) and under complete muscle relaxation. Three insulated 15cm needle-electrodes (NanoKnife, AngioDynamics, Latham, New York) with an exposure length of 20mm were placed along the long axis in the deepest part of the lesion under ultrasound and CT-fluoroscopy guidance, with an inter-electrode distance of exactly 20mm (figure 1C and 2). First, ten test-pulses of 1500 V/cm with a pulse length of 90μs were delivered for each electrode pair. After confirmation of correct delivered current (20-50 A) another 80 pulses were administered for each treatment vector. Next, to treat the superficial part of the tumor, another 90 pulses
minimal enhancement of 4.8 cm³, most likely representing residual tumor. Following multidisciplinary deliberation, the advice against pregnancy was withdrawn. A few weeks later she became pregnant. At 7 months gestation, tumor size had further decreased to 5.2 cm³ (figure 1H). At term, the patient had an uncomplicated vaginal delivery of a healthy son. In the postpartum period and in the months thereafter, no complications occurred.

Discussion

The optimal approach for a large hepatic adenoma in women with a desire to become pregnant has been topic of debate for quite some time. The risk of bleeding and rupture of these tumors during pregnancy and delivery represents a significant diagnostic and therapeutic challenge. Because of the low incidence of this tumor and subsequent lack of published evidence the exact risk is unknown, but increases significantly during the third trimester in which rising estrogen levels and increased hyperdynamic circulation can cause sudden growth of the adenoma. Postpartum, the risk is also high since the sudden withdrawal of estrogens after delivery can induce abrupt massive regression of the tumor, leading to hemorrhage. If rupture of an HCA during pregnancy does occur, it is associated with a reported maternal and fetal mortality rate up to 44% and 38%, respectively. The unpredictable behavior of hepatic adenomas during pregnancy has resulted in the disputable advice to either avoid or to undergo invasive treatment prior to pregnancy when adenomas exceed 5 cm or when complications occurred during a previous pregnancy.

Traditionally, surgical resection was the gold standard whenever invasive treatment was indicated, but during the past decades less invasive image-guided treatment options have gained increasing popularity. Percutaneous RFA is safe and effective and is associated with a short hospital stay. Selective arterial embolization can be used both as an elective treatment to reduce lesion size as well as the initial emergency treatment in case of active bleeding. The 5% chance of malignant transformation of hepatic adenomas may be considered an additional argument in favor of minimally-invasive treatment. Although promising reports have been published, the exact role of these strategies has yet to be determined. A watchful wait approach with periodical liver ultrasound may be considered for adenomas that are unsuitable for RFA, with trans-arterial embolization in case of hemorrhage.
In our case, both resection and thermal ablation were contra-indicated. Embolization had proved unsuccessful due to the tumor's extensive arterial blood supply. With this in mind we were particularly cautious towards the watchful wait approach, since emergency embolization in case of rupture would be extremely difficult.

The advantage of IRE is that it selectively destroys all cells within the ablation zone, whilst the supporting extracellular matrix structures consisting of collagenous and elastic fibers are preserved.\textsuperscript{5} As a result, large blood vessels and bile ducts within the portal triad who derive their strength and shape from these extracellular matrix structures remain patent, which is in contrast to thermal ablation techniques. Furthermore, treatment is not impeded by heat-sink, which suggests a potentially more effective treatment of tumor cells in close proximity to large vessels. With these distinctive characteristics, IRE has the potential to become a successful alternative ablation method for central liver tumors that are located near or around portal triad structures.\textsuperscript{13} The past years, several clinical studies have been conducted investigating the safety and efficacy of IRE for centrally located malignant liver tumors. Although the follow-up period is still short, results generally show a safe use of IRE near vessels and bile ducts,\textsuperscript{14,15} with a primary efficacy rate of 67-100%.\textsuperscript{16,17}

To our knowledge, IRE has not been previously used to treat benign lesions. Whereas for oncologic purposes complete tumor destruction is warranted, we only wanted to reduce the tumor size.\textsuperscript{. Therefore, we deliberately avoided ablation in the vicinity of the bile ducts. Based on studies on healthy porcine liver tissue the anticipated ablation zone usually extends 5mm outwards from the electrodes.\textsuperscript{18} However, on post-IRE CECT the ablated area proved significantly larger than we had anticipated, with the peripheral reactive rim still reaching the bile ducts (figure 1D). Local surroundings and tissue-specific conductivity both have an effect on size and shape of the ablation zone.\textsuperscript{20} Hypothetically, the increased ablation zone size could suggest that the conductivity of adenoma tissue is higher than that of normal liver parenchyma, resulting in a lower electric field threshold for IRE. Future work should correlate ablation volumes with numerical simulations to determine an effective electric field threshold for each particular tumor to guide future ablations.\textsuperscript{20}

In summary, this case suggests that when surgical intervention and thermal ablation of a large hepatic adenoma both carry a significant risk, IRE could be considered as an alternative treatment option. Although IRE is currently only used for the treatment of malignant tumors, the indication may also extend to specific benign tumors in the future.
IRE for hepatocellular adenoma

**Literature**

"Searching, seek and destroy
Searching, seek and destroy
Running, on our way
Hiding, you will pay

Metallica, Seek and Destroy"
Chapter 5
The Pancreas